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ANNUAL REPORT

Division of Intramural Research Programs
National Institute of Mental Health (U.S.)

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October 1, 1982 - September 30, 1983

VOLUME I SUMMARY STATEMENTS

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ANNUAL REPORT
of the
DIRECTOR, DIVISION OF INTRAMURAL RESEARCH PROGRAMS
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1982 - September 30, 1983

Frederick K. Goodwin, M.D.

My first full year (fiscal) as Director is summarized in this, my second annual report. Again, I am proud to have presided over another period of solid research productivity. Undaunted by continuing stresses and uncertainties, including an unexpected budget squeeze, our scientists have scored significant advances in their mission to understand and treat mental affliction. It has been a period of personnel adjustments and program redirection in the wake of last year's major reorganizations. For many of our promising younger investigators 1983 was the year of opportunity to step into well-deserved leadership roles. In some cases, this means new directions; in others new energy applied to old problems.

For starters, we should announce that our official status has changed from a "Program" to a "Division", although I will persist in using the former designation (albeit with a small "p") in this report. I initiated this change so that we might have a structure more in keeping with the NIH Institutes and the rest of NIMH.

Also, in recognition of her very special role that transcends that of an executive officer, I have elevated Hazel Rea to the position of Deputy Director. As Associate Director for Operations, Dr. Ted Colburn will assume some administrative responsibility and free Hazel to function as Deputy.

Again this year, the high quality of IRP research is reflected in a long list of awards received by our scientists. Dr. Giulio Cantoni was elected to membership in the National Academy of Sciences. Dr. Edward Evarts, already a member of the Academy, became a member of its Institute of Medicine, won the Carl Spencer Lashley Award, and the Presidential Rank Award. Dr. Irwin Kopin took first place in the Anna Monika Prize competition. In recognition of her outstanding leadership, Hazel Rea received DHHS's highest honor, the Distinguished Service Award for Administration. Dr. Robert Post received the APA's Foundations' Fund prize, Dr. Steven Paul was named one of the top 10 young Federal employees as a Fleming Award winner. Dr. Judith Rapoport received the APA's Ittleson Award. Drs. William Freed, Larry Siever and Thomas Uhde were all recipients of the A.E. Bennett Award. Drs. Werner Klee and Carolyn Waxler garnered ADAMHA Administrator's Awards for Meritorious achievement, and Dr. Richard Coppola won a Commissioned Corps Commendation Medal.

Many of these honors were conferred for specific research papers or a series of journal articles. As I mentioned last year, nine of our scientists rank among the 100 most frequently cited in scientific literature worldwide. On a per capita basis, this places NIMH IRP in a tie for second

place among its sister intramural programs on the NIH campus on this recognized index of scientific impact.

The continued preeminence of IRP science depends, in large part, on how skillfully we distribute our limited resources. One of the unique strengths of an intramural program such as ours, i.e., its potential for longterm stability in pursuing a particular line of investigation, must always be tempered by the parallel need for flexibility in pursuing other promising leads as opportunities present themselves. Over the past year, we have encouraged our Board of Scientific counselors to sharpen its scrutiny of ongoing projects to help us make the best possible decisions about research investments and priorities. They have proven extraordinarily effective in that regard.

During FY 1983 the Board reviewed the Clinical Neuroscience Branch, Clinical Psychobiology Branch, Biological Psychiatry Branch, Laboratory of General and Comparative Biochemistry, and Laboratory of Neurochemistry. The Laboratory Chiefs and I are now in the process of implementing a number of the Board's recommendations, which include some program redirections. A special panel of the Board reviewed a study proposal by Dr. Stan Greenspan, head of the Clinical Infant Research Program, which we acquired last year from the dismantled NIMH services program. The Infant Program's research orientation was deemed so different from that of the rest of the IRP that alternative placement within the DHHS is now being explored.

It has been especially gratifying to welcome as new regular members of the Board of Scientific Counselors this year Drs. Norman Garnezy, Daniel Freedman, and Herbert Weissbach, each a distinguished scholar in his own field and one a former mentor of mine. The other regular members of the Board are: Drs. Robert Moore, Anita Hendrickson and Brenda Milner.

Key personnel changes this year include the departures of Drs. Irv Kopin and Mike Ebert, the timely return of Dr. Seymour Kety, the arrival of Dr. Stephen Suomi, and the appointment of Dr. Rex Cowdry as Clinical Director.

Last summer Dr. Kopin moved just upstairs in the Clinical Center to become Scientific Director of our sister intramural program in the Neurology Institute (NINCDS). He had served NIMH, IRP for 26 years, most recently as Associate Director for Clinical Research and as Chief of the Laboratory of Clinical Science (LCS). Highlighting one of his many achievements, this past year Dr. Kopin and colleagues in the LCS announced development of the first good animal model of Parkinson's disease. His Anna-Monika prize mentioned earlier was for explication of the sources of catecholamine metabolites in CSF. We owe Dr. Kopin special gratitude for his tireless service as Associate Director during the leadership transition. Not the least of his accomplishments in this capacity was the management of last year's complex space shuffle occasioned by the opening of the ACRF. Dr. Kopin's stewardship of the Neurology program will reinforce the already close working relationship between the two major neuroscience programs on the NIH campus.

Succeeding Dr. Kopin as interim chief of the Laboratory of Clinical Science is our first scientific director Dr. Seymour Kety, who stepped down from that job to create the LCS and become its first chief 27 years ago.

Dr. Kety is also our new Associate Director for Basic Science, serving as my principle advisor on policy, management and planning matters relating to the basic components of the program. He will also continue some of his own research in neuropharmacology and genetic models of schizophrenia.

Dr. Ebert, who had been our clinical director, has left to become chairman of a unique jointly administered psychiatry/pharmacology program at Vanderbilt University. Over the past year, he brought distinction to himself and to NIMH by being named acting director of the Clinical Center. It is an honor and an historic milestone for a psychiatrist and NIMH staff member to serve as head of the nation's largest research hospital complex. Dr. Ebert's ascendancy to this albeit temporary high position was presaged by his election last year as chair of the NIH Medical Board.

I am very pleased to announce the appointment of Dr. Rex Cowdry as our new Clinical Director. During the past year and a half, as Associate Clinical Director for Outpatient Studies, Rex has skillfully managed the complex transition of programs into the new ACRF, at the same time initiating his own studies into the biology and pharmacology of borderline disorders. The selection of Dr. Cowdry capped a two month process during which several inside and outside candidates were carefully considered.

Within the LCS, we have already established a new section on Clinical Psychopharmacology under Dr. Potter to analyze drugs, neurotransmitters and their metabolites in body fluids. The new section's HPLC assays in tandem with the Analytical Biochemistry Section's GC-MS and other assays constitute the core of a central clinical biochemistry facility capable of an integrated assessment of a variety of important neurotransmitter substances in individual clinical samples.

The future research profile of the LCS's 3-East inpatient unit is now under careful review, a process which is being integrated with the work of our search committee for a new LCS chief. Among other things we plan to take advantage of the new understanding of stress physiology emerging from our basic labs--Dr. Axelrod's and others'--to expand clinical studies on stress. I have selected Dr. Dave Jimmerson to take over as acting Chief of the Section on Experimental Therapeutics, including administration of the 3-East unit.

LCS's new Section on Child Psychiatry has earned such a superior reputation that its Chief, Dr. Judy Rapoport, recently received a phone call--out of the blue--offering her an unsolicited half-million dollar grant from the Gulton Foundation. The money will be used over the next five years to support her research, including a variety of brain imaging studies on neuropsychiatrically disturbed children.

That work will be carried out in cooperation with the Section on Clinical Brain Imaging recently created within the Laboratory of Psychology and Psychopathology. The Section, with Dr. Robert Cohen as its chief, succeeds Dr. Monte Buchsbaum's old section in the BPB. It will work with both clinical and basic investigators in studies visualizing human brain function through Positron Emission Tomography (PET) and other developing imaging technologies.

The PET studies are poised to expand beginning in 1985 when the new NIMH PET scanner and the NIH cyclotron needed to make its radiolabeled compounds are scheduled to become operational. Our machine will be the prototype of a unique four ring design offering substantially better resolution than now available, much faster scans and shorter radiation exposure for subjects. The scanner and its companion high capacity computer and image processing system--which should prove useful in other applications as well--are being built for us by Scanditronix. We are grateful to staff of the NIH contracting office and to Dr. Rich Coppola, whose technical expertise proved an invaluable resource for the IRP in evaluating competing machines and negotiating this \$1.9 million contract over the past year.

Unfortunately, many other important equipment acquisitions had to be postponed in FY 1983 because of an unanticipated budgetary deficit resulting from our having to absorb the cost of pay increases and the new federal worker contribution to Medicare. These and other imponderables resulted in a \$1.5 million shortfall for IRP by the second quarter. We were able to weather this fiscal emergency only by the virtual cessation of any new hiring and equipment purchases. With impressive cooperation by the lab/branch chiefs and senior scientists, the austerity measures saved over \$1 million in less than half a year. We hope this kind of sacrifice will not have to be repeated; the FY 1984 budget should allow for some catching up. However, we remain concerned about our ability to keep our equipment up to date, given the continued inflation in scientific instruments and rapid obsolescence associated with high technology.

Returning to our roundup of personnel/organizational developments, I am proud to announce establishment of a unique naturalistic primate behavior laboratory at our animal facility in Poolesville, MD. Dr. Stephen Suomi, one of the world's leading investigators of primate behavior and until recently director of the Wisconsin Primate Laboratory, heads up this new unit, a joint venture of the NIMH and the Child Health Institute (NICHD). Dr. Suomi holds joint appointments as chief of NICHD's Comparative Ethology Laboratory and as Special Assistant to the Scientific Director, NIMH. Dr. Tom Insel along with Dr. Paul MacLean will coordinate NIMH projects at the new facility, which will draw on expertise from the various labs and branches. A naturalistic 3 acre environment for primates has been created around "Hazel's Pond" behind the Laboratory of Brain Evolution and Behavior buildings at Poolesville, and the first of what will eventually become a colony of 200 rhesus monkeys have already taken up residence. As far as we know, ours will be the first primate facility with the capacity to bring to bear both sophisticated pharmacological/biological techniques and behavioral/ethological approaches in the study of animal models of clinical illness. For the first time it will be possible, for example, to study effects on social behavior in uncaged animals of anxiety states induced by the new beta carboline compounds or models of stress and depression produced by infusions of corticotropin releasing factor. Individual, presumably genetic, differences in behavioral responses to environmental stress and separation might be correlated with neurochemical differences.

In working out the details of this joint undertaking over the past year

with NICHD's Scientific Director Dr. Art Levine, we agreed to provide the space and some positions while NICHD agreed to put up the money for purchase of animals, supplies and equipment, needed renovations of buildings and grounds, and the bulk of new positions needed to operate the facility. The primate behavior studies seemed a fitting successor to Dr. MacLean's and Dr. John Calhoun's pioneering research on primate behavior, brain development and social behavior of rodents, which have made the Poolesville operation something of a legend in its own time. Since the experimental phase of most of their work had ended, the time was ripe for a new generation of studies which, in a very real sense, will build on their legacy and provide deserved continuity to their work. Dr. MacLean will to stay on in Poolesville for a time to lend an experienced hand and nurture the fledgling research effort.

In other actions, we have elevated Dr. Elliot Gershon's old Section on Psychogenetics to a new Clinical Neurogenetics Branch. This unit conducts clinical and laboratory research on the role of genetic factors and gene-environment interactions in behavior, with special reference to psychiatric illness. Dr. Gershon's successful development of an investigative program is reflected in recent studies linking an inherited excess of muscarinic-cholinergic receptors in skin cells to major depressive illness, a finding which may prove to be a biological trait marker for depression.

Dr. Gershon's new branch and Dr. Mike Brownstein's Laboratory of Cell Biology established last year, along with other components of our program are investing in an emerging technology which holds special promise for understanding the brain: molecular genetics. By transplanting genetic material, animals with novel genes can be "mass produced" for physiological, pharmacological and behavioral studies. The effect of placing a neurotransmitter in nerve cells that do not normally make it can be investigated. Fundamental questions about how the brain, in all its complexity, grows from a small tube of primitive, undifferentiated cells can be pursued. Molecular genetics can also be applied to clinical problems. The causes of diseases that are genetically transmitted or that have major genetic components, including depression and schizophrenia, might be better characterized by studying the structure of patients' DNA and through other molecular approaches. In short, we have a unique opportunity today to make a quantum leap in our understanding of the nervous system. Unfortunately, new resources are required for a major thrust in this area. We are already on record with our request for these.

In the Biological Psychiatry Branch, Dr. Phil Gold has been made chief of a new Section on Neuroendocrinology to study neurohormonal and peptide alterations in the major psychiatric disorders. The critical role played by endocrine and neuropeptide factors in neural regulation and behavior, along with the now well-documented dysregulation of such factors in major depression warrant a heightened pace of investigation. Dr. Gold's preliminary studies over the past year using CRF as a challenge drug in both humans and primates suggest an excess secretion of that hypothalamic hormone in depression and forge a new link between stress and the etiology of depression. His presentation of this model was a highlight of an NIH-sponsored seminar for science writers in July on the "Molecular Basis of Stress" which I chaired and which also included presentations by Drs. Steve Paul and Julie Axelrod.

Also over the past year, we hosted our own NIMH science writer's seminar on memory (including a discussion of work in progress on Alzheimers Disease), featuring Drs. Herb Weingartner and Mort Mishkin, among others. Drs. Frank Putnam, Danny Weinberger, Stan Burns, Irv Kopin and Sandy Markey represented IRP in ADAMHA-sponsored science writers seminars.

Public education efforts have also included several tours of our labs for college groups and visiting professionals in the field and many, many TV, radio and print media interviews granted by our scientists, indicating a continuing high level of public interest in our work. This past year, we enlisted the help of the local print and broadcast media more systematically as a tool for recruiting research subjects.

The Director's Conference Series initiated last year to help promote interdisciplinary communication within our own program has continued to draw interested staffers to the ACRF amphitheater. Conferences were held in 1983 on neurotransmitter receptor function and chronobiology. I am also planning a series of smaller "Director's Seminars" beginning in early 1984 which will bring together small groups of basic and clinical investigators with a convergence of interests (of which they may not be fully aware) in a more intimate setting--the IRP library. The IRP also cosponsored an NIMH-wide Conference on Premenstrual Syndrome Research last spring, which was co-chaired by Dr. Dave Rubinow.

At this writing, the on-again/off-again planning authorization for an FDA building on the NIH campus, which would include space for that segment of our program now based at Saint Elizabeth's Hospital, seems to be off again--it did not make the Administration's 1985 proposed budget as we had hoped. Though this may put our plans for new space on a slower track, we remain hopeful that our days in the marginally adequate William Allen White Building will eventually end.

As I alluded to earlier, the FY 1984 research budget for NIMH allows for a modicum of growing room--a 12 percent increase. For this, we are indebted to the strong, effective advocacy for our program provided by Drs. Bud Mayer and Herb Pardes over the past year. Despite the prolonged uncertainties about the stability of leadership at the Agency and Institute levels--and Dr. Mayer's ultimate departure to the Pentagon--there was never a lapse in program morale. Again, our gratitude goes out to both of these able men.

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1982 - September 30, 1983

Robert M. Post, M.D., Chief

Major new outpatient studies have been initiated in the Branch that complement the ongoing studies of affectively ill patients on 3-West and the outpatient studies of the Section on Psychogenetics. Dr. Thomas W. Uhde has built an extensive outpatient program for the evaluation and treatment of patients with generalized anxiety and panic disorders. Patients are studied almost entirely on an outpatient basis with periodic short hospitalization for more intensive clinical or research evaluation. In addition to his work in the Unit on Neuroendocrinology headed by Dr. Philip Gold, Dr. David Rubinow has initiated studies of patients with menstrually-related mood disorders and also functions as Chief of the Consultation Liaison Service. Dr. Elliot Gershon, currently the Chief of the Section on Psychogenetics, will leave the Biological Psychiatry Branch to head up a laboratory-sized project on Psychogenetics. Drs. Paul Marangos and Jitendra Patel will join the Biological Psychiatry Branch to perform biochemical studies of clinical relevance and basic interest in relation to mechanisms underlying kindling and the mechanism of action of carbamazepine.

Unit on Anxiety and Affective Disorders (Dr. Thomas W. Uhde, Chief)

The Unit on Anxiety and Affective Disorders has been investigating the phenomenology, psychobiology, and pharmacology of primary anxiety disorders. Special attention has been given to agoraphobia and panic disorder which occur alone and as these symptom complexes which occur in association with major affective illness. The life course of illness has been defined in 30 patients referred for evaluation of panic attacks. Fifty-four percent of these patients have had symptoms of major depression at some time during their lifetime, although 20% had major depressive episodes prior to the onset of panic attacks. Major life events, i.e. separation/divorce, death of a relative, and geographical relocation, have occurred in approximately 80% of the patients within 6 months of the onset of panic attacks. First degree relatives appear to be at increased risk for alcoholism, anxiety, and depressive illness.

Biological correlates, particularly those reflecting noradrenergic functions, have been investigated in the series of patients with panic disorder. ³H-dihydroergocryptine platelet binding was increased, whereas prostaglandin E₁ stimulated cyclic AMP production was lower in panic patients compared to controls, suggesting the possibility of decreased function inspite of increased receptor binding. The growth hormone response to clonidine also appears to be blunted in patients with panic disorder, suggesting decreased alpha-2 adrenergic receptor function. In panic disorder patients, both the platelet data and growth hormone response to clonidine, studied in collaboration with Dr. Marion Kafka, showed abnormalities that were similar to patients with major affective illness compared to controls. They also showed a shortened REM latency. In contrast, other biological indices associated with major affective illness were not observed in patients with panic disorder. Thus, patients with panic disorder did not display a decreased number of platelet tritiated imipramine binding sites, or abnormal escape from dexamethasone suppression. Together, these data suggest that patients with panic disorder and major affective illness

share some similarities in relation to noradrenergic function, but that other biological markers separate the two syndromes. These findings are of particular interest in relation to the common responses in these two syndromes to treatment with tricyclic and monoamine oxidase inhibitor antidepressant regimens.

Dr. Uhde has demonstrated that clonidine, an alpha-2 adrenergic agonist, has anxiolytic properties in depressed and anxious patients. In contrast, yohimbine, an alpha-2 antagonist, induces panic attacks in patients with panic disorder. Studies with yohimbine may provide an advantage over other agents such as lactate which has been used as a provocative test in anxious patients, but whose mechanism of action is less clearly specified than that of yohimbine. The preliminary data also suggest that patients with panic disorder, compared to normal controls or affectively ill patients, are especially vulnerable to the anxiogenic effects of caffeine based on questionnaire data. Studies are in progress to document this observation with double-blind administration of caffeine to anxious patients and controls. Initial data suggest that caffeine has dose-related anxiogenic properties in normal volunteers.

In addition to the noradrenergic system, preliminary data are also suggestive of an important role for benzodiazepine and adenosine systems in the mediation of normal and pathological anxiety. Several double-blind trials have been initiated to assess the effects of alprazolam, carbamazepine, clonidine, and imipramine treatment on symptoms of generalized anxiety and the incidence of panic attacks. Preliminary data suggest that each of these medications may be useful in subgroups of anxious patients.

The Unit on Neuroendocrinology (Dr. Philip Gold, Chief)

The Unit on Neuroendocrinology is being elevated to a Section level, consistent with an increased recognition of the importance of neuroendocrine and peptide regulation of normal and pathological affect and behavior and in accordance with Dr. Gold's leadership role in this area. This emphasis is also consistent with the enormous explosion in basic science information and the elucidation of putative peptide neurotransmitters in brain. Capitalizing on one of these recent developments, Dr. Gold in collaboration with Dr. Loriaux and his investigative group have begun to explore the recently-sequenced corticotropin releasing hormone (CRF) in nonhuman primates and in man. CRF in cynomolgus macaques produces prolonged dose-dependent increases in ACTH and cortisol reflecting hypothalamic-pituitary-adrenal activation as well as dose-dependent increases in growth hormone and prolactin; these responses, but not the ACTH or cortisol responses, are modified by the administration of agents which influence either the endogenous opiate or monoaminergic neurotransmitter systems. The plasma half-life of CRF in primates and humans is longer than for any other known hypothalamic releasing factor. Intracerebroventricular CRF administration also results in responses classically associated with stress such as dose-dependent increases in plasma catecholamines and cortisol. CRF is cleared from the intracerebroventricular space more rapidly than substances cleared by bulk flow.

CRF (1 µg/kg i.v.) has been administered to normal volunteers, patients with major psychiatric disorders, and subjects with endocrine disease at 2000 h. The normals, in contrast to the chaired-stressed non-human primates, failed to show growth hormone or prolactin responses, but showed significant increases in ACTH,

beta-endorphin, and cortisol. However, drug-free patients with affective illness showed a significant GH response to CRF. Moreover, preliminary evidence in patients with bipolar affective disorder show that subjects in the depressed phase show a significant attenuation of the ACTH and cortisol responses to CRF. Following a constant infusion of CRF for 24 hours in normal volunteers, ACTH and cortisol rose slowly over three hours and plateaued for the remainder of the infusion at levels similar to the magnitude of hypercortisolism seen in depressed subjects. In patients with Cushing's disease, there was a robust ACTH response to CRF despite high baseline cortisol levels, indicating a very significant defect in feedback regulation at the pituitary. The findings in depression and Cushing's disease suggest that the loci of dysfunction in the HPA axis in these two entities is different, and that the CRF test may aid in the differential diagnosis of these disorders.

Studies of the posterior pituitary hormones, oxytocin and vasopressin, are also continuing in affectively ill subjects with anorexia nervosa and controls. Evidence suggests an altered ratio of these compounds in CSF in mania compared to depression, with relative increases in vasopressin and decreases in oxytocin in the manic compared to the depressed phase of the illness. CRF fails to elevate vasopressin secretion in human and nonhuman primates. In primates, Dr. Gold has demonstrated that a glucocorticoid receptor antagonist elevates plasma vasopressin, confirming previous indirect suggestions that glucocorticoids play a role in the regulation of tonic vasopressin secretion.

The Unit on Peptides and Consultation Liaison Service (Dr. David Rubinow, Chief)

Working in conjunction with the Unit on Neuroendocrinology, Dr. Rubinow is studying normal levels and diurnal variation of a variety of endocrine and putative neuropeptide neurotransmitters in the CSF of affectively ill patients and controls. He is also assessing the effects of oxytocin and vasopressin on mood, endocrine function, and memory in patients and controls. He has established a major outpatient initiative to study the behavioral, biochemical, and physiological concomitants of menstrually-related mood disorders. He has developed consistent rating methodologies and found that only 40% of the first 60 women self referred and reported to have menstrually-related mood syndromes showed clear evidence for this disorder. Prospective ratings of the syndrome and its precise relation to menstrual phase is clearly required in order to describe subgroups, follow the course of the illness, and to investigate treatment response. Over 500 women have requested participation in the project to date and are in various stages of evaluation. Clinical and biological study of patients with clear onsets and offsets of mood and behavioral disturbances associated with menstrual phase should help illustrate critical mechanisms underlying this disorder and related affective disorders.

Dr. Rubinow has also initiated an extensive series of scientific investigations based on the development of the clinical psychiatric consultation service to the other institutes in the NIH Clinical Center. Thus, this service should not only provide a critical clinical consultation to the other institutes, but should provide an expanding and novel format for research on a variety of disease models and clinical medical-psychiatric interfaces.

Unit on Behavioral Pharmacology (Dr. Agu Pert, Chief)

Dr. Agu Pert's work focuses on interactions of classical and neuropeptide

effects on behavior, response to acute and repeated stress, and elucidation of the mechanism of action of psychotropic drugs such as lithium and carbamazepine. The techniques of in vivo autoradiography for visualization of changes in opiate receptor occupancy following behavior manipulations have been conducted in collaboration with Drs. Thomas Seeger and Candice Pert. Following cold water stress in the rat, decreased ^3H -diprenorphine binding was observed in many caudal subcortical structures, while cortex, hippocampus, and more rostral structures were largely unchanged. The greatest decreases (up to 60%) were seen in brain areas such as periaqueductal gray, reticular formation, and the midline-intralaminar nuclei of thalamus, reflecting opiate peptide release in areas which receive and modulate incoming pain information. Lesser decreases in binding were seen in hypothalamic and limbic areas such as the amygdala, the bed nucleus, and preoptic area. In a footshock control group, decreases were similar but less extreme. This in vivo autoradiographic technique holds great promise both for the study of other behaviorally induced changes in patterns of opiate release and for the eventual adaptation application to visualization of other putative neurotransmitter systems in brain.

The effects of neurotensin on dopaminergic activity following intranigral intrastratial injection were examined in collaboration with Hilleary D. Everist. Neurotensin induced a pattern of dopaminergic alterations suggesting that it turned off dopaminergic activity in the nigrastratial system in a manner similar to axonal transection or treatment with gamma-butyrolactone.

Using $2\text{-}^{14}\text{C}$ deoxyglucose techniques, pathways showing increased glucose utilization during self-stimulation of brain have been identified in collaboration with Dr. R.U. Esposito. In collaboration with Dr. Henry Holcomb, it has also been observed that stimulation of substantia nigra in animals pretreated with haloperidol increased glucose utilization in the ipsilateral globus pallidus, entopeduncular, and subthalamic nucleus compared to nontreated, stimulated animals. This technique and 6-OHDA lesions are used to assess the extent that nigral dopamine neurons contribute to the pattern of metabolic activation. Initial findings suggest that elevated pallidal glucose metabolic activity following nigral stimulation is due either to the effects of dopaminergic denervation or stimulation of non-dopaminergic fibers.

Section on Psychogenetics (Dr. Elliot Gershon, Chief)

The Section on Psychogenetics has as its goals: (1) identification of biological and psychological factors that are genetically transmitted in major psychiatric disorders and (2) pharmacologic and pharmacogenetic studies of psychoactive agents in normal controls.

Biological genetic studies form a core focus of the program. Continued studies of the fibroblasts as a clinical model for neuronal characteristics reveal: (1) affectively ill patients and ill relatives have increased muscarinic receptor density, compared to normal controls; (2) lithium produces decreased muscarinic receptor density in fibroblasts, with greater reduction in fibroblasts of patients than controls; (3) choline acetyltransferase activity is present in fibroblasts, suggesting further similarity between fibroblasts and cholinergic neurons; (4) beta-adrenergic receptor density is decreased in patients and ill relatives compared to controls; (5) specific binding was absent for a series of ten other neurotransmitter and neuropeptide receptor ligands.

Other trait markers of affective disorders are being sought in studies of ambulatory euthymic drug-free bipolar patients as compared with normal volunteers. For example, plasma GABA is significantly decreased in euthymic bipolar patients. Pedigree studies of plasma GABA are underway to determine if this heritable characteristic is a genetic trait marker.

A major initiative is in progress in the molecular genetics laboratory component of the Section in an attempt to study proopiomelanocortin (POMC). A restriction fragment length polymorphism of POMC has been identified. Further studies of polymorphisms of POMC, and of their physiological significance, are underway.

An "at risk" longitudinal study of adolescent and young adult offspring of bipolar patients has been undertaken with the focus on biological markers in psychosocial development in these individuals. Theoretical analysis of the power of biological comparisons in at-risk groups has allowed the genetics group to reject certain putative markers for study based on their statistical distribution. A family study of schizophrenia has also been initiated. A family study of anorexia nervosa has been completed, firmly establishing the link between anorexia and affective illness in that a high incidence of affective illness was observed in the relatives of patients with anorexia nervosa. The family studies of the Section have established substantial methodological advances demonstrating that diagnostic information from relatives requires an average of four informants per individual for minimal acceptability of family history data.

Section on Psychobiology (Dr. Robert M. Post, Acting Chief)

Work in collaboration with Dr. Thomas W. Uhde continues to document the clinical efficacy of carbamazepine in the acute and prophylactic treatment of manic and depressive patients. Plasma and CSF levels of the active metabolite of carbamazepine (carbamazepine-10,11-epoxide) are better correlated with degree of clinical response than levels of the parent compound. Carbamazepine has a multiplicity of effects on classical neurotransmitter and putative neuropeptide systems. Studies are underway to explore which of these effects is related to carbamazepine's clinical profile of side effects or therapeutic efficacy in paroxysmal pain syndromes, affective illness, and seizure disorders. Carbamazepine has interesting contrasting effects on several systems, compared to lithium carbonate, which are of clinical and theoretical import. In contrast to lithium carbonate, which produces the diabetes insipidus syndrome, carbamazepine appears to have agonist-like effects on vasopressin function. Carbamazepine may thus be of particular clinical utility in the treatment of patients unable to tolerate lithium because of renal side effects. Carbamazepine also significantly reduces, while lithium elevates, peripheral white blood counts. In contrast to classical neuroleptic agents which block dopamine receptors, substantial evidence indicates that carbamazepine does not act by directly affecting this dopamine receptor system. Thus, maintenance treatment with this agent has not been associated with the development of acute or chronic neurological syndromes such as Parkinsonian side effects or tardive dyskinesia, and thus may prove a useful adjunct or alternative to maintenance neuroleptic treatments in some patients. Carbamazepine decreases somatostatin in CSF, findings of particular interest in relationship to the observation by others that amygdala-kindled seizures produce long-lasting elevation in brain somatostatin in the rat. These findings are also of interest in light of

observations of Dr. Rubinow that somatostatin is significantly decreased in the cerebrospinal fluid of depressed patients compared to euthymic or manic patients or normal controls. Recent work in collaboration with Drs. Marangos and Patel reveals that carbamazepine has potent effects at adenosine receptors displacing binding of tritiated cyclohexyladenosine and diphenylxanthine with high potency; however, the ability to displace binding of either of these ligands is not closely associated with the anticonvulsant efficacy of a series of 12 carbamazepine analogs. These data suggest that carbamazepine may have important effects on adenosine receptor systems, but raise the question of whether its anticonvulsant properties may not be mediated by effects at other neurotransmitter systems. Carbamazepine displaces binding at the peripheral benzodiazepine receptor marked by [^{3} H]-RO5-4864 more potently than at the classical or central benzodiazepine receptor marked by [^{3} H]-benzodiazepine. In conjunction with this biochemical data, physiological evidence, in collaboration with Dr. Susan Weiss, indicates that RO5-4864 reverses carbamazepine's anticonvulsant effects on amygdala-kindled seizures while the antagonists RO15-1788 and CGS-8216 which act at the central site are ineffective. Thus, these biochemical and physiological data are consistent in suggesting the possible important role of the "peripheral" benzodiazepine receptor site in the mechanism of anticonvulsant action of carbamazepine.

Studies in collaboration with Dr. Frank W. Putnam, now in the Adult Psychiatry Branch, continue to document important behavioral, autonomic, electrophysiological, and cognitive alterations across different personalities in patients with multiple personality syndrome.

Dr. Gerald Brown is continuing his studies and initiating new ones in order to study neurotransmitter alterations in patients with suicidality and aggression. Several studies have documented findings of low levels of the serotonin metabolite 5-hydroxyindoleacetic (5HIAA) in patients with histories of aggression and/or suicidal behavior. Dr. Brown has replicated his earlier finding that urinary MHPG excretion has decreased in a time dependent fashion following amphetamine. Tryptophan, administered in a single dose to hyperactive children, produces expected alterations in the ratio of plasma amino acids and appears to have similar clinical effects to a single dose of d-amphetamine. D-amphetamine administered for two weeks has been shown to have an anti-aggressive effect on hyperactive children in whom conduct disorder was prominent.

Annual Report of the Clinical Neuropharmacology Branch
National Institute of Mental Health
October 1, 1982 to September 30, 1983

Dennis L. Murphy, M.D., Chief

Most of the research questions of the Clinical Neuropharmacology Branch come from the area of clinical problems. We systematize our investigations in patients by beginning with an initial evaluation period using standardized behavioral and biological assessment measures. We then use experimental interventions, most often neurotransmitter/neuromodulator-selective drugs, as probes of the responsivity state of particular brain neurotransmitter systems, with special interest, of course, in agents with therapeutic potential. Our current emphasis is on the development and testing of better ways to assess specific elements of brain function in man which might provide insight into biological factors in psychopathologic states.

This preamble helps introduce some current developments within the Branch. The group has diverged into new clinical problem areas over the past 1-3 years. In addition to continuing to study some patients with primary affective disorders, we reported last year the rapid growth of our studies of adult obsessive-compulsive patients led by Dr. Thomas Insel. We also noted the beginnings of a project centered around individuals with severe premenstrual psychopathology, developed by Dr. Jean Hamilton. This year, as the obsessive-compulsive disorder and premenstrual syndrome disorder projects became predominantly outpatient rather than inpatient studies (taking advantage of the new ACRF clinic), we have begun to admit inpatients with presenile dementia (Alzheimer's disease), as part of a study devised by Dr. Robert M. Cohen.

While this range of diagnoses might seem unreasonably broad for our small band of clinician-investigators, our venturesomeness was presaged by detailed studies over the past decade of depressed patients which uncovered, within the heterogeneity of this disorder, many types of character pathology including some severe obsessional states, as well as many manifestations of severe cognitive and memory problems, panic symptoms, premenstrual exacerbation of affective symptoms, and anxiety as a major component of the core depressive symptomatology.

The decision to extend our patient studies to these disparate diagnostic groups was also based on collaborative studies with other groups using single doses of pharmacologic agents like clonidine (as part of Dr. Larry Siever's work) to evaluate the responsivity of noradrenergic receptor-mediated functions such as the changes in blood pressure, growth hormone and cortisol produced by the drug. This approach provided new insights into similarities and differences among patients with depression, anorexia nervosa and bulimia, panic disorder, and obsessive-compulsive disorder, and, importantly, into changes in these responses produced by long-term treatment with antidepressants. As noted below, obsessive-compulsive patients manifested a consistent pattern of alterations in a series of biological markers generally regarded as linked to major depressive disorder alone.

Affective symptoms, cognitive problems and other phenomena which we have been studying are prominent in some patients with obsessive-compulsive disorder, the premenstrual syndrome, and patients with Alzheimer's disease, and thus part of our strategy is to better characterize so-called "secondary depression" and other related "secondary" psychological and biological features in these patient groups. At the same time, we hope our specialized, non-mainline investigational approaches may illuminate some new features of the "primary" disorder. All of our studies involve collaborators from other NIMH Branches and NIH Institutes, so that other available study opportunities with these patients are not neglected.

It needs to be mentioned that an important architect of the pharmacologic challenge strategy within the Branch, Dr. Larry Siever, has been sorely missed since he departed last year to carry on a program of his own in the Department of Psychiatry at Mt. Sinai Medical School and the Bronx VA Hospital. Dr. Siever and I organized and co-chaired a recent symposium which will be published in toto dealing with the treatment aspects of the cross-diagnostic paradigm we are pursuing, entitled "Therapeutic Responses to Tricyclic Antidepressant Drugs in Non-Affective Disorder Patients." Dr. Siever's work in the Branch was honored by his receiving the A.E. Bennett award for young investigators given by the Biological Psychiatry Society. Dr. Edward Mueller has taken on some of the pharmacologic challenge work in the serotonergic system area, and Dr. Trey Sunderland has been developing some new cholinergic system challenges with Dr. Cohen. Dr. Siever has been able to return several days a month during this transition time to help in the completion of some of his original projects.

We also are going to be missing the full time efforts of Dr. Jean Hamilton, who has joined the Center for Studies of Affective Disorders in the NIMH extramural program, but will continue some of her work in the Branch part-time. Dr. Robert Cohen is also formally leaving the Branch in that he has been named to head up the Clinical Brain Imaging Section in the Laboratory of Psychology and Psychopathology, and his major contributions to our clinical and laboratory efforts as well as to the overall scientific scholarship of the Branch will leave a large gap in the group. Fortunately, he is just around the corner and keeps a very important and influential hand in almost all of his Branch projects.

Specific research advances within the Branch's programs have been in several areas, which reflect not only our primary clinical research orientation but also our belief that the best clinical science is done in an atmosphere where every clinician-investigator also has opportunities to develop and use laboratory assays or animal models in parallel with the clinical studies.

Adaptational processes in neurotransmitter systems during long-term treatment with antidepressant drugs. Our group's clinical studies with the monoamine oxidase-inhibiting antidepressant, clorgyline, had provided indirect evidence that noradrenergic system changes, as reflected in cerebrospinal fluid, plasma and urine amine and amine metabolite changes, were more closely correlated with antidepressant effects than comparable measures in serotonin, dopamine or trace amine systems.

Using several rodent model preparations, Dr. Cohen confirmed his earlier work with our previous visiting scientist, Dr. Iain Campbell, indicating that brain α_1 -, α_2 - and β -adrenoceptors were altered by long-term clorgyline

treatment at doses very close to those used clinically, while short-term treatment was ineffective. The new studies demonstrated that during chronic clorgyline treatment, norepinephrine release produced by KCl stimulation in a rat brain microsac preparation was markedly increased. Microsacs prepared from animals treated with clorgyline for 21 days also showed a marked decrease in the inhibition of norepinephrine release normally produced by the selective α_2 -adrenoceptor agonist, clonidine. These functional changes in norepinephrine release were accompanied by a >50% reduction in brain stem α_2 -receptor density as measured by [^3H]-clonidine binding. Also at 21 days, but not at three days, clorgyline treatment was associated with a significant blockade of clonidine's normal suppressant effects on locomotor activity in the rat, an apparent behavioral concomitant of the biochemical alterations.

These results thus provided direct physiological support for a change in norepinephrine release mechanisms and noradrenergic receptors during long-term clorgyline treatment. Dr. Charanjit Aulakh, working with Dr. Cohen, has begun to explore some more specific behavioral alterations during acute and chronic treatment with clorgyline, as well as during the withdrawal period following clorgyline treatment. Using the self-stimulation paradigm in rodents with electrodes implanted in several brain areas, some similar adaptive changes were observed, specifically an attenuation of the usual suppressant effect of clonidine on self-stimulation behavior.

Dr. Cohen and coworkers within the Branch had previously proposed that an overly stringent negative feedback system could impair the capacity of the catecholamine pathways to convey information adequately in depressed patients. The present data, particularly those pertaining to reinforcement mechanisms known to be involved in self-stimulation behavior, support the possibility that some antidepressants such as clorgyline might act to reset intra-synaptic communication and feedback mechanisms as a component of their molecular mode of action.

It has long been recognized that complete therapeutic responses to antidepressants do not occur immediately, but require several weeks of continued drug administration, even in the case of the MAO-inhibitors, where full treatment doses are started immediately. We have previously documented time lags in the development of rapid eye movement sleep suppression after beginning clorgyline administration, and even longer delays in the time of onset of one quite direct measurement of adrenoceptor responsiveness, the hypotensive response to single doses of clonidine after 3 days, 10 days and 21 days of continued clorgyline treatment of depressed patients. This year, Dr. Benjamin Roy completed a detailed analysis of the sequence of cardiovascular changes which occurred during clorgyline administration to 17 depressed patients. Clorgyline treatment was accompanied by a gradual reduction in systolic and diastolic blood pressures and a slowing in pulse during the first two weeks of treatment. During the third and fourth weeks of treatment and the first post-treatment week, mean arterial blood pressure reductions became maximal, together with relatively greater reductions in standing than recumbent blood pressure, i.e. the development of orthostatic hypotension. During the last two weeks of clorgyline administration, pulse rate increases elicited by standing failed to increase further with the greater fall in standing blood pressure occurring at this time, suggesting an alteration in this regulatory mechanism.

Reductions in pulse and diastolic blood pressure during clorgyline administration were significantly correlated with depression rating reductions. Pretreatment systolic and diastolic blood pressures and pulses were significantly correlated with blood pressure reductions in the fourth week of clorgyline treatment, as well as with clinical improvement as indicated by reductions in depression ratings on the Hamilton and Beck scales. Decreases in plasma norepinephrine and in plasma and urine 3-methoxy, 4-hydroxy phenylglycol (MHPG) concentrations suggested that the blood pressure reductions and clinical antidepressant responses produced by clorgyline were accompanied by reductions in central sympathetic outflow.

These clinical data closely resemble the changes in brain noradrenergic metabolism and receptor adaptation following clorgyline in animals which suggest a downregulation of central noradrenergic functioning during treatment with this antidepressant. This interpretation is in keeping with the revised hypotheses for depression invoking dysregulation of catecholamine functions and, in one version, postulating an overactive, inefficient state of catecholaminergic synaptic function in endogenously depressed patients. Some direct evidence in favor of this view comes from data published this year with Dr. Ray Lake indicating that our depressed patients as a group have highly significant plasma norepinephrine elevations in comparison to well-matched controls. The functional state of the central noradrenergic system might alternatively be interpreted as being reequilibrated by the changes produced by antidepressant drugs, perhaps establishing a new, more beneficial steady state. While our evidence is quite preliminary, these analogous studies in animals and patients illustrate the potential for illuminating possible mechanisms of action of antidepressant drugs in humans, and further suggest the importance of examining and correlating the sequential development of biological and behavioral changes during the course of longer term antidepressant drug treatment.

Neurotransmitter and neuroendocrine studies in non-human primates. Another model used in parallel to some of our clinical investigations is that provided by the opportunities to readily sample cerebrospinal fluid and examine biochemical-behavioral relationships in rhesus monkeys. Some important information regarding prolactin, β -endorphin and cortisol interactions in cerebrospinal fluid compared to plasma have proved of value in our studies in humans using pharmacological challenge strategies with neuroendocrine endpoint measures.

In addition, attempts to measure serotonin in cerebrospinal fluid to evaluate antidepressant drug effects on serotonergic mechanisms led to the discovery of a very large diurnal rhythm in serotonin, with nighttime peaks 20-fold higher than daytime values. The diurnal pattern followed that of melatonin, and both the serotonin and melatonin rhythms were equally suppressed by continuous light and by the β -adrenergic antagonist, propranolol, indicating the likelihood of a common regulatory mechanism.

Psychobiology and treatment response study of obsessive-compulsive disorder. The comprehensive investigation of individuals with obsessive-compulsive disorder patients directed by Dr. Thomas Insel has completed its first generation of studies and is beginning a new series of projects. Thus far approximately 35 patients with this uncommon, treatment-resistant disorder from

all over the U.S. have entered the program. Early psychobiological results suggested a surprising similarity between these patients and patients with affective disorders. Studies of dexamethasone suppression tests, sleep physiology, and receptor sensitivity all revealed biologic abnormalities similar to those seen in depression, even though the obsessive-compulsive patients do not regularly show a disturbance of mood. Initial results from a family study found a higher than expected number of relatives with affective disorders and depression spectrum disorders (i.e. alcoholism as well as depression). In addition, psychological testing revealed the presence of obsessional characteristics in a subgroup of apparently well parents of the patients. A related family study report emphasizing family interrelationships is being completed by Dr. Carol Hoover.

Eighteen obsessionals were studied using a series of neuropsychological tests to examine suggestions of an "organic" basis for this unusual disorder. Although many of the individuals had difficulty with a spatial memory task, no overall pattern suggestive of neurological deficits was found. In addition, cerebral tomography scans (obtained in a subgroup of 10 patients with suggested abnormalities on either neuropsychological testing or routine EEG examinations) revealed no difference in ventricular or cortical size between obsessional patients and age-matched controls.

In a carefully controlled treatment study, 13 patients were randomly assigned to either the tricyclic antidepressant, clomipramine, or the selective MAO inhibitor, clorgyline. The study was designed as a crossover comparison of the two drugs, so that each patient would at some point receive both compounds and thus serve as his own control. Clomipramine was found to be consistently, although modestly effective in reducing ratings of obsessions, depression, and anxiety (assessed by a "blind" observer). By contrast, clorgyline administration was not associated with statistically significant improvement on any of the rating scales. As several of the obsessional patients had secondary depressions and many (even without depressive symptoms) had sleep EEG abnormalities similar to those found in depression, we considered that the clinical improvement following clomipramine administration may have been an antidepressant effect. This, however, seems unlikely as those patients with neither the clinical nor the biological features of depression consistently improved on clomipramine. In contrast, clorgyline, which has been previously demonstrated to be an antidepressant in primary affective disorder patients, was not effective for the obsessional symptoms. Clomipramine thus may have an anti-obsessional effect independent of its role as an antidepressant. As clomipramine is a powerful inhibitor of neuronal serotonin uptake, and plasma levels of clomipramine (but not its less serotonin-selective metabolites) were significantly correlated with the reduction in obsessional symptoms, serotonin may play an important role in the drug-induced reduction of obsessional symptoms.

To follow up on this intriguing lead, we have launched a new series of studies to investigate the role of serotonin in obsessive-compulsive disorder. Studies underway in untreated obsessionals include platelet ^3H -imipramine binding and platelet serotonin uptake measurement, cerebrospinal fluid 5-hydroxyindoleacetic acid determinations, and an examination of the neuro-endocrine responses to the serotonergic agonist, fenfluramine. A new treatment study is comparing the effectiveness of zimelidine, an antidepressant which selectively inhibits serotonin uptake, to desipramine, which predominantly blocks the reuptake of brain norepinephrine and not serotonin.

Affective symptoms associated with the menstrual cycle. About 130 subjects have now taken part in various parts of a series of investigations initiated by Dr. Jean Hamilton on menstrual cycle-related affective symptomatology. In one substudy, human plasma β -endorphin was shown to be relatively stable through the menstrual cycle. In contrast to predictions of an association between premenstrual symptomatology and endogenous opioid withdrawal put forward by Reid and Yen, our data indicated that when symptoms were associated with plasma β -endorphin changes, the symptomatology accompanied a rise in β -endorphin rather than a decline. This question is being examined further in cerebrospinal fluid collected at intervals during the menstrual cycle, and by pharmacologic challenges using naloxone.

In another study carried out with Drs. Sanford Markey and Lee Poth, Dr. Hamilton was able to demonstrate significant group differences in 6-hydroxymelatonin excretion when individuals were separated according to high and low levels of premenstrual symptomatology as rated on the Moos Scale. Melatonin may have an inhibitory effect on ovulation in women, and the suggestion of seasonal alterations in melatonin may be pertinent to the findings of variations in both depressive mood and of menstrual cycle length according to time of the year.

Follow-up neuroendocrine and behavioral data were also obtained from a screening survey of normal volunteers who could be separated into relatively high and low premenstrual symptom groups, and compared with another group seeking help for premenstrual syndrome difficulties. The symptomatic premenstrual group in the community differed in having a higher incidence of atypical depression (increased sleep, appetite, anger, sexuality) than the low premenstrual syndrome group. A great deal of data from both the clinical and screening studies remains to be analyzed, including items pertinent to life-events and concurrent daily stress ratings, other personality measures, and clinical histories and questionnaires regarding family and personal psychiatric history.

Use of biochemical and biophysical techniques to study metabolism and storage of monoamines. Dr. Jonathan Costa has utilized a variety of biochemical and biophysical techniques to explore mechanisms in the metabolism and storage of monoamines in model systems, platelets, and other types of amine storing tissues.

Electron microprobe analysis has been used to measure the elemental composition of storage granules in bacteria and platelets. Bacterial storage granules appear to contain polyamines which may be displaced by divalent cations, and their staining properties may play an important role in determination of bacterial genera in certain cases. Platelet storage granules have a core whose mass thickness appears to be independent of the membrane surface area of the granule; these granules accumulate basic amines to a similar extent regardless of their surface area. X-ray microscopy of both conventionally-prepared and hydrated specimens reveals novel cytoskeletal structures which may play a role in the formation of bacterial storage granules and in the exocytosis of platelet storage granules. Structure-function relationships suggested by this technique may be resolved by application of Synchrotron x-ray microscopy or x-ray holography to similar specimens.

Nuclear magnetic resonance has been employed to explore the utilization of 2-fluoro-deoxyglucose in the rat brain and to assess more critically the nature of the monoamine storage complex in nerve-ending particles (microsacs), chromaffin vesicles, and platelets from several animal species and humans. In all storage vesicles examined to date, the ring moiety of both catecholamines and indoleamines appears to assume motional properties similar to both the ring and phosphate groups of intravesicular nucleotides. The tumbling rate of the nucleotides is determined in turn by the concentration of divalent cations and the relative extent of hydration of the core constituents. Higher magnesium concentrations and relative osmotic dehydration favor the formation of aggregates which are viscous (gel-like) at lowered temperatures, while higher calcium concentrations lead to precipitation and consequent immobilization of all vesicle constituents. Nevertheless, even when vesicle contents assume a solid-like state, intravesicular lithium appears to rotate freely, suggesting that the aqueous portion of the vesicle interior is mobile. The motional state of bacterial storage granules containing polyphosphate and polyamines exhibits similar sensitivity to divalent cations.

Conventional biochemical techniques, including application of radiolabelled tracers, atomic absorption measurements, polarographic techniques, and enzyme assays have been utilized to extend our understanding of the mechanism of action of tricyclic antidepressants and of amine metabolism, storage, and transport. Elucidation of the role of phenolsulfotransferase in amine disposition in platelets would be facilitated if an irreversible inhibitor of the enzyme were available, and several putative candidates are currently being examined. Although one site on the platelet enzyme has a relatively high affinity for dopamine, normetanephrine, and difluoroserotonin, a second site with lower affinity may process other, non-amine substrates under physiological conditions. Tricyclic antidepressant drugs act at micromolar concentrations to uncouple energy metabolism and inhibit ATPase activity and electron transport in isolated mitochondria and submitochondrial particles. In eukaryotic cells with a proton-pumping ATPase in the plasma membrane, tricyclic antidepressants inhibit the ATPase and act to collapse the transmembrane pH gradient. These actions may provide a rationale for the effects of tricyclic antidepressants on ATPase- or proton-pumping-associated uptake of amines at the plasma membranes of mammalian cells. Other work suggests an important role for membrane lipid fluidity (or some other temperature- and drug-modulated conformational change in a cell membrane component) both in maintaining vesicular amine stores and in permitting uptake of amines across cell plasma membranes.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Thomas A. Wehr, M.D., Acting Chief
October 1, 1982 through September 30, 1983

The Clinical Psychobiology Branch directs its primary research effort towards understanding the spectrum of affective and sleep disorders and their treatment. A major theme of research in the Branch is the role of biological rhythms in the pathophysiology of these disorders. The sleep-wake cycle and other circadian rhythms are generated by a clock-like pacemaker in the hypothalamus. The intrinsic rhythm of this pacemaker, about one cycle every 25 hours, is in turn controlled by periodic stimuli that function as environmental time cues (zeitgebers). Zeitgebers reset the phase of the pacemaker so that its rhythm is adjusted to one cycle/24 hours and it maintains a characteristic phase relationship to the external day-night cycle; otherwise the timing of sleep and other circadian rhythms would occur one hour later each day and gradually drift around the clock. In animals light-dark transitions (dawn, dusk) are the most important zeitgebers. Part of the research mission of the Clinical Psychobiology Branch (CPB) is to increase our understanding of the mechanisms that regulate the normal human circadian system and to elucidate its role in the pathophysiology of affective and sleep disorders in both of which the timing and other features of circadian rhythms have been shown to be abnormal. Clinical experiments have shown that procedures that alter the timing of the sleep-wake cycle and other circadian rhythms can induce recurrences in both affective and sleep disorders. For this reason we believe the role of circadian rhythm disturbances in the pathogenesis of these conditions may be critical and deserves careful investigation.

EFFECTS OF LIGHT ON BIOLOGICAL RHYTHMS

There is ample reason to believe that environmental light plays a primary role in the regulation of the timing of human circadian rhythms. Light is the principal zeitgeber in all animal species investigated. Work elsewhere has shown that human circadian rhythms can be entrained to light-dark cycles alone and that the range of entrainment is a function of light intensity. The retino-hypothalamic tract and the suprachiasmatic nucleus of the hypothalamus mediate both entrainment of circadian rhythms and suppression of nocturnal melatonin secretion by light. Work in our laboratory has shown that human melatonin secretion can be suppressed by light and that the degree of suppression is a function of light intensity.

We are investigating hypothalamic effects of light in several ways. We are refining our methods for measuring light suppression of nocturnal melatonin secretion. Light will be administered in a more quantitative manner using a xenon light source, infrared and ultraviolet filters, fiberoptic light guides and ganzfeld-type goggles. Light intensity will be regulated with neutral density filters and can be expressed as watts $\cdot$ cm⁻² retina. Using this equipment a fluence-response curve can be generated in which degree of melatonin suppression is expressed as a function of log light intensity. (Melatonin levels will be measured by our collaborator Alfred Lewy, M.D., Ph.D., at the University of Oregon. It is our hope to establish a radioimmunoassay for melatonin in the Branch's laboratory.) Other projects are designed to investigate the role of light in the entrainment of circadian rhythms. In animals whose circadian rhythms are free-running in constant darkness, discrete light pulses cause phase-shifts in the timing of the rhythms. The amount and direction of shift is determined by when in the course of the animal's circadian rhythms the light pulse is presented.

From such experiments it is possible to construct a phase response curve (PRC) in which degree and direction of phase shift is expressed as a function of the circadian phase at which the light pulse was presented. Some pulses cause advances, some delays, and some have no effect. We are attempting to generate a human PRC. Normal subjects live in near darkness in isolation from external time cues for two weeks. During the course of the experiment they are exposed to a single six-hour pulse of high intensity light (our previous experiments showed that the threshold for hypothalamic effects of light is very high in humans). Circadian rhythms in motor activity, sleep EEG, rectal temperature and behavioral events are monitored continuously. Phase shifts in these rhythms after bright light pulses will be used to generate a PRC. Entrainment of circadian rhythms by light will also be explored by measuring the effect of changes in timing of light-dark cycles on the phase position of the melatonin circadian rhythm.

Our previous work showed that in blind subjects the melatonin rhythm exhibited abnormal phase relationships to the day-night cycles or, in 50% of the cases, was actually free-running and no longer entrained to it. This evidence strongly suggests that light serves to entrain the melatonin rhythm to the day-night cycle in humans. In further experiments, while healthy subjects are entrained to a normal sleep-wake schedule they will be exposed to eight hours of bright light either early or late in the day. After one week on either lighting regimen, 24-hour profiles of melatonin secretion will be obtained in constant dim light (to prevent masking of the melatonin rhythms by bright light). Early light pulses are predicted to advance the timing of the melatonin rhythm compared with late pulses.

CIRCADIAN RHYTHMS AND AFFECTIVE DISORDERS

In affective disorders our group and others have described abnormalities in the phases, amplitudes, and waveforms of circadian rhythms. Changes also occur in the timing and duration of sleep and sleep stages. Experiments described below suggest that some of these changes play a key role in pathogenesis of depression.

Clinical work in the Branch focuses on longitudinal studies of spontaneous changes in sleep and circadian rhythms during the course of affective episodes and on experiments designed to evaluate clinical effects of enforced changes in the timing of sleep and circadian rhythms. In the first type of study, wrist motor activity, sleep EEG rectal temperature and clinical state are monitored continuously through one or more affective episodes. Patients with a rapid cycling form of manic-depressive illness are most suitable for this type of study. In such patients we have found that the timing and duration of sleep exhibit dramatic state-dependent changes. For example, when patients switch out of depression into mania they often exhibit single or consecutive 48-hour sleep-wake cycles. We found that one night's total sleep deprivation, which simulates a 48-hour sleep-wake cycle, is capable of causing such patients to switch from depression to mania. Therefore, 48-hour sleep-wake cycles that occur spontaneously at the time of the switch from depression to mania may help to cause the switch. The occurrence of 48-hour sleep-wake cycles in patients may depend on a mechanism that is latent in normal circadian physiology since healthy subjects sometimes spontaneously exhibit near 48-hour sleep-wake cycles in temporal isolation experiments.

Our recent experiments provide further evidence that the timing of other circadian rhythms is abnormal in manic-depressive illness. The rectal temperature rhythm

is sometimes normal and sometimes shifted earlier than normal in the same patient. Thus its phase position is (1) variably advanced and (2) unstable. Phase advanced circadian rhythms could help to explain why the temporal distribution of REM sleep is advanced in depression, with short REM latency and variably long first REM sleep episodes. We are investigating the possibility that the REM sleep circadian rhythm is phase-advanced in depression by recording sleep EEG for one-hour each three-hours for 48 hours in patients and controls. In this way a 24-hour profile of REM sleep propensity can be obtained. We hypothesize that the timing of the REM sleep propensity circadian rhythm is shifted several hours earlier than normal in depressives with characteristic REM sleep abnormalities, such as short REM latency. We are also explaining the possible relationship between REM sleep abnormalities and rectal temperature rhythm abnormalities by recording REM sleep changes in normal subjects in whom core body temperature is controlled externally by a computer regulated "spacesuit."

It seems likely that abnormalities in the timing of the sleep-wake cycle and circadian rhythms are important in the pathogenesis of depression because experimental manipulations of the timing of these cycles sometimes lead to dramatic changes in the clinical state of patients. Previously we showed in uncontrolled studies that shifting the timing of the sleep-wake cycle six hours earlier than usual (sleep from 5 pm to 1 am, instead of 11 pm to 7 am) could induce clinical remissions in depressed patients for 10 or more days. We are now completing a study in which early sleep (9 pm to 2 am) is compared to late sleep (2 am to 7 am). In 12 patients we have shown that shifting sleep earlier has antidepressant effects while shifting it later does not. This result is consistent with our previous uncontrolled observations and with work of other investigators, and seems to indicate that the timing of sleep relative to that of other circadian rhythms can control the clinical state of affective patients. Specifically, there appears to be a critical early morning circadian phase when sleeping or waking determines clinical state. Depression appears to be (1) sleep-dependent and (2) requires that sleep coincide with a critical circadian phase. Events occurring during this critical phase (2 am to 7 am) may hold the key to mechanisms underlying the pathogenesis of depressive illness. In order to follow this clue, current and future projects in the Branch are designed to further delimit the formal properties of antidepressant sleep manipulations and to document their biological correlates in sleep physiology, neurotransmitter metabolism and neuroendocrine function.

In principle abnormalities in the circadian systems of patients with affective disorder could arise from the rhythm generating mechanism (e.g. SCN) or the mechanism by which it is entrained to zeitgebers (e.g. RHT). Both possibilities are being investigated. In preliminary studies we showed that manic-depressives may be supersensitive to hypothalamic effects of light (suppression of MT). In the future we hope to confirm these findings with better quantitative methods, by generating light-melatonin suppression fluence-response curves in patients, as described above. Eventually, it may be possible to investigate entrainment mechanisms directly by measuring a PRC as described above. The rhythm generating mechanism is being investigated by monitoring wrist motor activity, sleep EEG, rectal temperature, behavioral events and clinical state in patients living in isolation from external time cues. To date, four patients have been studied. In one patient, the rhythm generating mechanism appeared to run abnormally fast (a possible cause of phase-advanced circadian rhythms under conditions of entrainment). Additional patients will be studied.

SEASONAL RHYTHMS AND AFFECTIVE DISORDERS

Seasonal rhythms in behavior and physiology occur in many species. Fundamentally there are two types of seasonal rhythms: circannual rhythms, which are endogenous and persist in the absence of cyclic annual external factors and non-circannual rhythms which are driven by annual changes in day length (the photoperiod). The latter also depend on the circadian pacemaker complex to (1) register light (via RHT) and (2) measure its daily duration (via SCN).

Previously we identified a subgroup of patients with an annual rhythm of affective episodes: winter depressions and spring hypomanias. Depressions begin in November and are characterized by hypersomnia, weight gain, carbohydrate craving, and typical depressive symptoms such as social withdrawal, depressed mood, decreased energy and reduced self-esteem. Several biological correlates have been investigated. Slow-wave sleep and conception rates are reduced by 50% in winter. This year these findings have been replicated in a second group of patients. Considering that animal annual rhythms are regulated by light, we hypothesized that patients' depressions were triggered by changes in the daily photoperiod. Our first experiments showed that winter depressions could be ended by exposing patients to days which were artificially lengthened with bright artificial light. Bright artificial light was effective whereas light of ordinary intensity was not (presumably because the human hypothalamus is relatively insensitive to the latter). Fortunately because of the relatively high threshold for light, light of ordinary intensity has proven to be a plausible placebo for the light treatment. This year in a second group of patients we confirmed that light is an effective treatment and ruled out the possibility that partial sleep deprivation (a necessary consequence of the early morning light treatments and a known effective treatment modality) contributed to the antidepressant effect.

The pineal gland and its hormone melatonin play a central role in the regulation of animal photoperiodic annual rhythms. Pinealectomy abolishes these rhythms, apparently because the duration of nocturnal melatonin secretion, in effect, encodes the duration of night and transmits this information to target organs involved in regulation of seasonal behaviors. This year we will investigate the possible role of the pineal in winter depression by attempting to reverse the therapeutic effect of light with exogenous melatonin. We do not yet know if seasonal affective disorder is an endogenous type circannual rhythm or an exogenous type photoperiodic annual rhythm. In animals photoperiodic rhythms can be controlled by two light pulses per day (skeleton photoperiod). If the interval between pulses is short, the animal responds as if it were winter. If the interval is long (e.g. more than 12.5 hours) a summer response is elicited. In both cases the total light exposure is the same; it is the timing of the light that is critical. Apparently light in long skeleton photoperiods interacts with a critical photosensitive phase of the circadian system to trigger summer responses. Patients with winter depression will be exposed to long and short skeleton photoperiods in isolation units in order to determine whether or not seasonal affective disorder depends on a photoperiodic mechanism, and whether the timing of light rather than its total amount is critical for the therapeutic response.

Our investigations of the antidepressant effects of sleep schedule shifts and light have converged on similar models in which light and sleep act on critical early morning circadian phases. It is reasonable to ask whether these two modalities are in fact fundamentally related to one another. As described above, we have determined that partial sleep deprivation is not sufficient to explain

antidepressant responses to light treatment. In another study patients were totally sleep deprived on one night in very bright light and on another night in almost total darkness. The antidepressant effects of sleep deprivation were the same in both conditions. Thus antidepressant effects of manipulations of the timing of light and sleep appear to be fundamentally independent (though they may act through a final common pathway).

CIRCADIAN RHYTHMS AND SLEEP DISORDERS

Approximately 15% of patients consulting sleep disorder clinics suffer from insomnia caused by abnormal timing of the circadian pacemaker. In the delayed sleep phase syndrome (DSPS) the timing of the sleep-wake cycle is shifted several hours later than normal. Patients have difficulty falling asleep until late at night, sleep normally once asleep, and have difficulty arising in the morning. Patients who have ordinary jobs suffer sleep deprivation during the week and sleep long hours on weekends to compensate. DSPS is a disorder of the timing, not the quality of sleep. In principle the disorder could arise from two causes: (1) an abnormally slow rhythm-generating mechanism (slow pacemakers entrain to the day-night cycle in a more delayed phase position than fast ones); (2) an abnormal phase-response curve for light (the advance part of the curve may be relatively insensitive to light or the delay part supersensitive. These possibilities will be investigated by (1) measuring the intrinsic period of the rhythm generating process in temporal isolation experiments and (2) measuring a PRC for light in selected patients. Hypothalamic sensitivity to light will also be assessed by obtaining a fluence-response curve for melatonin suppression by light in DSPS patients.

BIOLOGICAL RHYTHMS AND PREMENSTRUAL SYNDROME

Premenstrual syndrome (PMS) resembles rapid cycling manic-depressive illness and seasonal affective disorder in that (1) it is cyclic, and (2) it occurs in women. A project in progress in the Branch is designed to explore this analogy by determining whether sleep EEG patterns exhibit cyclic changes in PMS and whether manipulations of the sleep-wake cycle ameliorate PMS symptoms. This latter possibility is particularly interesting because excessive prolactin secretion has been implicated in PMS and prolactin secretion is known to be sleep dependent.

FUTURE DIRECTIONS OF RESEARCH

Basic research with animals has revealed that three known hypothalamic effects of light (circadian pacemaker phase-resetting, suppression of melatonin secretion, and annual photoperiodic responses) can be mimicked by nicotinic cholinergic agonists. It seems likely that our various experimental uses of light will lead to novel pharmacological approaches to treatment of seasonal depression, jet lag, shift work and delayed sleep phase syndrome.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Irwin J. Kopin, M.D., Chief
October 1, 1982 through September 30, 1983

Introduction

The various Sections and Units of the Laboratory of Clinical Science have continued to conduct independent programs of research. These programs often involve collaborations within the Laboratory and with investigators in other institutes or outside of the NIH. The major activities of the programs are directed toward defining mechanisms regulating the development and function of the nervous system, understanding the role of the nervous system in maintaining bodily functions, internal environment and mental state and determining the molecular events underlying drug and hormonal actions. Clinical studies are designed to define abnormalities and explain mechanisms of disturbed brain or other neurological functions, thereby providing rational bases for approaches to therapy of neuropsychiatric disorders.

There have been leadership changes in the Intramural Research Program and the administrative structure of the Laboratory will be changing. Younger members of the staff will be promoted to positions appropriate to their degree of scientific stature; the research program, however, will be able to proceed undisturbed and continue to evolve in accordance with the most promising directions as perceived by the individual investigators. A portion of the Laboratory has been transferred to a new laboratory under the direction of Dr. Michael J. Brownstein. The new laboratory consists of Drs. Martin Zatz, Miklos Palkovits and Fusao Hirata as Section Chiefs.

Dr. Michael Ebert, Chief of the Section on Experimental Therapeutics has been appointed Clinical Director, NIMH and will continue in a dual role.

A number of staffing changes have occurred or will soon be implemented.

Dr. Judith Rapoport has joined the Laboratory as Chief, Section on Child Psychiatry. Mrs. Edna Gordon, Chief of the Unit on Clinical Biochemistry, retired after having been with the Laboratory of Clinical Science for about 25 years. Her expertise will be missed. Her unit has been incorporated into other sections of the Laboratory; Dr. Sanford Markey is Chief of a new Section on Analytical Biochemistry and Dr. William Potter is to head a section which will include a central biochemical facility.

Dr. Irwin Kopin, Chief of the Laboratory of Clinical Science also served as Associate Director for Clinical Research in the Intramural Research Program. He has accepted a position as Director, Intramural Research Program, National Institute of Neurological and Communicative Disorders and Stroke. Present plans are for Dr. Seymour Kety to assume a dual position of Chief, Laboratory of Clinical Science and Associate Director, Intramural Research Program, NIMH. Dr. Kopin will continue as Chief, Section on Medicine for at least one year, allowing a smooth transition.

The Anna Monika Foundation awarded its 1983 first prize for research in the biological basis of depression to Dr. Irwin J. Kopin. Dr. Judith Rapoport received the only pediatric psychiatry research award given by the American Psychiatric Association, the Ittleson Prize, for 1983.

The following summaries of the year's activities have been provided by the Section Chiefs of the Laboratory:

SECTION ON PHARMACOLOGY
Julius Axelrod, Ph.D., Chief

Using AtT-20/D16-16 mouse pituitary cells rich in corticotrops we found that there are many hormones that can stimulate the release of ACTH. These are corticotropin releasing factor (CRF), catecholamines and vasoactive intestinal peptide (VIP). Catecholamines release ACTH via a β_2 -adrenergic receptor mechanism. The receptor mediated release of ACTH by these hormones is a result of generation of cyclic AMP. ACTH liberates glucocorticoids, which inhibit the CRF, catecholamine and VIP mediated release of ACTH in AtT-20/D16-16 cells. Somatostatin also blocks the receptor-mediated release of ACTH by blocking the action of adenylate cyclase.

Pretreatment of AtT-20/D16-16 cells with a β -adrenergic agonist markedly reduces the release of ACTH by catecholamines. A similar desensitization of ACTH release by CRF in primary pituitary cells was found. A desensitization of the inhibitory effects of somatostatin to receptor mediated ACTH was observed. We have found that isoproterenol can cause the secretion of ACTH in the rat by directly stimulating the β_2 -adrenergic receptors on the anterior pituitary. The catecholamine-stimulated release of ACTH in vivo can be blocked by glucocorticoids. These observations show that the three stress hormones, catecholamines, ACTH and glucocorticoids, are interrelated and should have important implications in understanding different types of stress.

Enzymatic carboxyl methylation reduces negative charges on proteins and alters their structure and properties. This reaction is associated with exocytotic release of neurotransmitters which is also dependent on depolarization of the cell membrane and Ca^{++} movements. Posterior pituitary hormones vasopressin and oxytocin and their corresponding neurophysins are released by exocytosis. Brattleboro rats which lack vasopressin have an animal version of diabetes insipidus. Posterior pituitaries of these animals are deficient in endogenous methyl acceptor proteins, mostly as a result of the absence of vasopressin-associated neurophysin. Protein carboxyl methylase activity is high in Brattleboro rats, probably related to hyperactivity of the neurosecretory posterior pituitary system, suggesting a close association between protein carboxylmethylation and posterior pituitary neurosecretion.

Catecholamines in postero-intermediate pituitary vary in a circadian rhythm and regulated by estrogen receptors. In addition to catecholamines, serotonin and histamine participate in the central regulation of cardiovascular function. There are two distinct enzymes in the brain which are capable of synthesizing epinephrine. These enzymes are not distributed similarly. In addition to hypothalamic and brain stem catecholamines, septal catecholamines participate in the central stress response.

Spontaneously hypertensive rats show alterations in angiotensin converting enzyme in peripheral tissues and specific forebrain nuclei. Changes in somatostatin metabolism and in the activity of angiotensin converting enzyme occur in pituitary gland and specific brain nuclei of Brattleboro rats.

Endogenous somatostatin occurs in the pituitary gland, where it is probably present in nerve terminals radiating from the hypothalamus to the intermediate lobe. In the intermediate lobe, somatostatin inhibits the isoproterenol-stimulated adenylate cyclase, probably altering release of neuropeptides from this structure.

SECTION ON HISTOPHARMACOLOGY David M. Jacobowitz, Ph.D., Chief

The research in this section has focused on peptidergic, monoaminergic and cholinergic neurons in the central nervous system. Immunocytochemical and neurochemical characterization of peptide-containing nerves in the brain and periphery has been pursued. Histochemical and biochemical analyses are carried out at the level of discrete central nuclei and pathways in order to define the underlying basis for behavioral changes and human pathology.

A new program was instituted to study unusual proteins in discrete regions of the brain. Such proteins can be isolated, injected into rabbits and the antisera generated used to immunocytochemically localize neuronal subpopulations. Different neuronal types can act as markers showing features of the organization of the CNS.

Using two-dimensional gel electrophoresis (2DE), the location (on polyacrylamide gels) and relative concentration of a number of different proteins from 25 distinct neuroanatomical regions of the male rat brain were examined. Some proteins were found to vary widely (greater than 10-fold) in concentration among the different brain regions. Repeated administration of the tricyclic antidepressant desmethylimipramine (DMI) from the hippocampus and parietal cortex caused a reduction in the apparent concentration of two proteins, whereas reserpine administration caused an apparent increase in the concentration of these proteins in the hippocampus, but no change in the parietal cortex. A third protein was elevated in concentration in the hippocampus by repeated DMI administration and reduced in concentration by repeated reserpine administration. Acute drug administration was found to be without effect. Since chronic DMI and reserpine cause down and up regulation, respectively, of the β -adrenergic receptors, these proteins may be part of the catecholamine receptor (Drs. Heydorn, Goldman, Merrill and Jacobowitz).

We continue our ongoing interest in mapping the localization of brain neuropeptides as antisera become available. Using an antibody to synthetic ovine CRF, CRF immunoreactivity was observed in the hypothalamus, thalamus, amygdala, cerebral cortex, midbrain, pons, medulla and spinal cord, but was not in the peripheral nervous system. CRF was found to coexist with substance P and acetylcholinesterase (AChE) in cells of the nucleus tegmenti dorsalis (ntdl). These CRF-SP-AChE cells were found to project to the medial frontal cortex, septum and thalamus. Immunocytochemical studies also revealed the co-localization of substance P (SP) and AChE within discrete loci in the interpeduncular nucleus of the brain. Muscarinic receptors (H^3 -QNB ligand) and α -bungarotoxin binding sites (nicotinic receptors) had distributions

resembling that of AChE. Lesions of the habenulae did not change the number or distribution pattern of the receptors. A morphological basis for the previously suggested modulation of nicotinic responses by SP was provided by the co-localization of SP and AChE. One physiological function of AChE in the ip may be to hydrolyze SP, thus terminating its biological activity (Drs. Olschowka, Rotter and Jacobowitz).

Pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers in the rat central and peripheral nervous systems are widely distributed. A number of PP-immunoreactive cells were demonstrated to coexist with catecholamine neurons in the pons-medulla and in a subpopulation of superior cervical ganglia neurons which innervate the blood vessels of the head and neck. Following electrical stimulation of the cervical sympathetic trunk, PP was co-released with norepinephrine from the nerves. The release of PP seems to be reserpine resistant. All PP-immunoreactive neurons disappear following 6-OHDA treatment. A neuromodulatory role of PP in autonomic functions was suggested by the effects of PP of several species on the isolated rat vas deferens. Neuropeptide Y (NPY) was also studied because of its sequence homology to the pancreatic polypeptides. The contractile responses, which were mediated predominantly by activation of noradrenergic nerves following electrical stimulation, were inhibited by bovine (BPP), human (HPP), and avian pancreatic polypeptide as well as by NPY, presumably by a presynaptic inhibitory mechanism on noradrenergic nerve terminals (Drs. Olschowka, Diz).

Immunohistochemical studies of 3-MSH revealed a distribution of immunoreactive neurons limited to the arcuate nucleus and basomedial hypothalamus. These cells were demonstrated to also contain α -MSH (Dr. Olschowka).

Studies were undertaken to reveal the physiological significance of a variety of neuropeptides in the brain. The effects of discrete intrahypothalamic injection of α -MSH on blood pressure and heart rate were observed in halothane anesthetized rats. Injection of α -MSH (0.6-1.2 nmol) into the rostral dorsomedial hypothalamic nucleus but not other areas resulted in an increase in heart rate which was accompanied by a slight increase in blood pressure. Tachyphylaxis to the response was apparent for at least three hours following initial exposure to the peptide. Studies on the central cardiovascular (CV) effects of other neuropeptides suggest multiple sites of CV activity within the hypothalamus, which is in agreement with electrical stimulation data.

Dermorphin and TRH produced increases in HR with minimal increases in blood pressure (BP) when injected into the anterior hypothalamic nucleus (AH). In addition, TRH causes increases in BP and HR from sites in the preoptic supra-chiasmatic nucleus (POS), a response which appears to be due to a more general activation of the sympathetic nervous system and may include vagal withdrawal as the sympathetic component of the increased HR. TRH has modest tachycardic effects in parts of the medial preoptic nucleus (POM) and in the DM and posterior hypothalamic nucleus (PH). Bradykinin resembles TRH in its site-dependent actions; increases in BP and HR in the DM and PH, tachycardia in the POS and POM and bradycardia in the PVN. Importantly, with each peptide, the CV responses were obtained in areas of the hypothalamus that have been reported to contain receptors and/or immunocytochemical evidence of fibers and cells containing the peptide. However, not all hypothalamic areas containing a given peptide responded with CV changes when the peptide was injected. All peptides

tested had a relatively long duration of action (40 min to 90 min) and tachyphylaxis to repeated injections developed. These findings suggest a possible role of these peptides in the central control of cardiac action. (Diz).

Work within the Unit on Neurochemistry under the direction of Dr. Paul Marangos has proceeded in three different areas including studies relating to the adenosine receptor, protein phosphorylation and clinical studies relating to the neuronal enolase and other brain specific proteins. Some of the progress made within the last year is summarized below.

In brain, adenosine receptor subtypes exist which preferentially recognize either the agonist or antagonist. A photoaffinity probe for the adenosine uptake site has been developed and is currently being used to examine the molecular structure of this site. After chronic caffeine administration adenosine receptors are increased. These studies strongly suggest that the anxiogenic effects of caffeine may be due to a hyperfunction of the adenosine system.

The brain specific S-100 protein has been found to be a potent inhibitor of protein phosphorylation. Several soluble and membrane-associated proteins are affected by S-100 in a manner reciprocal to that of calmodulin. The S-100 protein therefore appears to play a key role in brain protein phosphorylation, and this protein may represent a brain specific "calmodulin type" agent that is involved in receptor linked effector mechanisms.

Studies utilizing the neuron specific enolase (NSE) have focused primarily in the clinical area. We have shown this year that of 130 pediatric neuroblastoma patients, 96% had highly elevated serum NSE levels. The NSE serum level was also highly predictive of survival time, indicating that serum NSE levels may be of prognostic relevance in this disorder. NSE immunocytochemistry is also proving highly useful in monitoring transformed cells in the bone marrow of neuroblastoma patients. This has caused excitement, since it offers a way of determining whether treated bone marrow ready for reimplantation is truly cancer free and can greatly improve the outcome of this type of treatment.

SECTION ON MEDICINE Irwin J. Kopin, Chief

Fluorinated catecholamine derivatives were examined with the aim of developing false adrenergic transmitters which could be labelled with ^{18}F and used in Position Emission Tomographic or Nuclear Magnetic Resonance studies of adrenergic activity in vivo. 6-Fluorodopamine was demonstrated to be taken up into sympathetic neurones, converted by dopamine- β -hydroxylase to 6-Fluoronorepinephrine (6F-NE) which is stored and released by sympathetic nerve stimulation in the same manner as is endogenous norepinephrine. Thus, 6F-NE is a false adrenergic transmitter which can be used as a valid tracer in studies of noradrenergic function in humans.

Reflexes which modulate sympathetic nerve activity confirmed studies of the relationships between the effects of nerve stimulation and the physiological or plasma catecholamine responses. This can be avoided by using pithed rats in which the sympathetic outflow can be stimulated without reflex responses. Using this technique, the pressor responses and corresponding plasma norepinephrine levels during sympathetic stimulation as norepinephrine infusion can be compared. This more effective blockade by α_1 -adrenergic antagonists of pressor responses to stimulation and the neater susceptibility to α_2 -adrenoceptor blockade of pressor responses to infused norepinephrine led to the concept that α_1 -adrenoceptors are inside the neuroeffector junction whereas α_2 -adrenoceptors are mainly extrajunctional. The effects of drugs, such as cocaine and desipramine which block neuronal uptake of norepinephrine suggest that the uptake site is between the α_1 and α_2 adrenoceptors.

Inhibition of uptake potentiates markedly the pressor effects of administered norepinephrine after α_2 -adrenoceptor blockade but not the effects of stimulation. Although the pressor effects of sympathetic stimulation are not greatly enhanced by inhibition of uptake, overflow of norepinephrine into the circulation is markedly increased (about 3-fold).

Tyramine releases norepinephrine from sympathetic nerves, but also inhibits uptake of the catecholamine. The relationship of plasma norepinephrine to the degree of blood pressure elevation during the pressor effects of tyramine is similar to that of sympathetic stimulation after uptake is inhibited by DMI, suggesting that tyramine and sympathetic stimulation release norepinephrine from the same sites.

The cardiac output and its distribution to the various tissues has been examined in pithed rats before and during pressor responses to sympathetic stimulation and infused norepinephrine. Opposite effects of circulating vs. endogenously released norepinephrine may indicate differences in accessibility to α_1 receptors, different receptors mediating the responses. This appears most prominently in the splanchnic vascular bed.

Studies in intake animals complement those in pithed rats and provide information about central nervous system regulation of cardiovascular function. Prostaglandin $F_{2\alpha}$ injected into cerebral ventricles increases heart rate, blood pressure, respiration, and body temperature. Sympathetic as well as renal-angiotensin responses appear to be involved in this effect. Opiates injected into the cerebral ventricles of awake rats cause increased sympathetic activity which appears to be mediated by μ -receptors.

Vasopressin, angiotensin, and norepinephrine released from sympathetic nerves all play a role in reflex mediated maintenance of blood pressure. All are blocked by leukotriene D_4 , one of the major components of "slow reacting substance" released during anaphylactic shock.

The changes in dopamine receptor number which occur when dopamine release is blocked appear to be attenuated by lithium treatment. This may be important in the treatment of affective disorders or in management of tardive dyskinesia.

SECTION ON ANALYTICAL BIOCHEMISTRY
Sanford P. Markey, Ph. D., Chief

Melatonin: Studies on the role of melatonin and its metabolites in normals have been continued by Drs. Markey, Sadayoshi Higa and Merrily Poth. Dr. Higa has measured daily urinary 6-hydroxymelatonin excretion of six normal women over two continuous menstrual cycles. There is no evident correlation between the amplitude of the pineal hormone metabolite formation and the state of the menstrual cycle, although marked day-to-day variations exceeding those observed in normal men were observed. In order to determine whether the daily variations in 6-hydroxymelatonin excretion are an accurate reflection in pineal gland synthetic activity, a study has been initiated to correlate hourly plasma melatonin levels, assayed by RIA, with urinary melatonin metabolite levels.

Dr. Higa has developed an assay for 5-methoxyindoleacetic acid in animal and human urine, and has found that it is excreted at a constant rate, unlike the marked circadian variation exhibited by 6-hydroxymelatonin. Men and women excrete similar quantities (5-10 $\mu\text{g/day}$). Although melatonin injected into rats enhances the excretion of 5-methoxyindoleacetic acid, the endogenous metabolite does not derive from pineal melatonin. Pinealectomized rats excrete amounts similar to intact animals. Thus, although 5-methoxyindoleacetic acid would be a simpler compound to assay for pineal gland function studies, it fails to demonstrate the specificity of 6-hydroxymelatonin.

Longitudinal studies of melatonin production during pubertal development are continuing. A group of young girls has been studied every several months during the past several years. Urinary 6-hydroxymelatonin assays have thus far indicated a constant pattern of melatonin production during the early stage of pubertal development using Tanner criteria.

Drug Metabolism: Drs. R.S. Burns, S. Markey, C. Chieuh and I.J. Kopin, have resumed studies on the neurotoxic compounds related to the reverse ester of meperidine. Previously, the neuropharmacological effects of several compounds which had been synthesized and implicated in the induction of a parkinsonian syndrome in a young drug abuser were studied. Extending these observations to monkeys, a specific and persistent neurotoxicity affecting the nigral striatal dopamine pathway in primates, and not the adjacent mesolimbic dopamine system was demonstrated. The neurochemical effects of this comparison are described in the report of the Section on Experimental Therapeutics. The monkey model of Parkinson's disease is being pursued as a means of testing new therapeutic agents for human use, for determining the molecular action of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP), and for determining whether this chemical neurotoxicity has relevance to the underlying mechanisms of idiopathic Parkinson's disease. In the Section on Analytical Biochemistry, stable and radiolabelled compounds related to the neurotoxin have been synthesized and their metabolic fate is being studied in several animal species. Curiously, NMPTP does not produce a similar neurotoxicity in the rat, guinea pig, or cat in comparison to that observed in the primate. Differences in metabolism of NMPTP are being used as a possible indicator of species susceptibility to the neurotoxicity.

Dr. Jan Johannessen has pursued studies on the mechanism of morphine tolerance transferral between addicted and naive rats. The observation that cerebrospinal fluid from addicted rats blunts the analgesic effect of morphine when injected intraventricularly into naive rats has been replicated. Chemical characterization of the principle(s) responsible for this activity in cerebrospinal fluid has begun to determine whether the effect is due to an endogenously produced species or a morphine metabolite.

Instrumentation: Dr. Markey in collaboration with Dr. Fred Abramson has continued the development of a nuclide specific detector for gas chromatography utilizing a microwave plasma discharge interface between a capillary gas chromatograph and a mass spectrometer. During the past year, two systems have been refined and preliminary testing has been initiated. In one system, organic compounds eluting from the chromatographic column are reacted with oxygen in the microwave discharge to produce CO or CO₂, which can be detected with high sensitivity at m/z 28 or 44. Carbon isotopes from labelled species can be detected at m/z 29 (13C), 30 (14C), etc. An alternative system utilizes a hydrogen discharge to convert organic compounds to CH₄. High resolution mass spectrometry is required to separate the carbon isotopes from background signals from other gases (i.e. H₂O). Ultimate sensitivities around 1-10 dpm for 14C are expected. The systems will be tested in the coming year with several drug metabolism problems requiring the detection of isotopically species in complex mixtures.

Dr. Leonid Kelner (BEIB) working with Dr. Markey has completed an experimental surface ionization mass spectrometer which is currently being tested for its analytical capabilities. Intact conjugates of drugs, phospholipids, neuropeptides, and other polar compounds are being analyzed to test the applicability of fast atom and quantitative determinations of these compounds in complex biological fluids or tissue extracts.

SECTION ON EXPERIMENTAL THERAPEUTICS Michael H. Ebert, M.D., Chief

Clinical research in the Section on Experimental Therapeutics has focused this year on neurochemical, neuroendocrine, and pharmacological studies of several psychosomatic and neurological syndromes in which disorders of neurotransmitter metabolism are suspected. Studies of eating disorders (anorexia nervosa and bulimia) have expanded and studies of Korsakoff's psychosis, Parkinson's disease and essential tremor continued.

Clinical studies of anorexia nervosa have continued to focus on neurochemical disturbances that occur during the evolution of this disease (Drs. Kaye, Gwirtsman, Ebert). A clinical design is utilized in which we study anorectics while underweight, throughout weight recovery and at intervals after weight restoration. In collaboration with Drs. Gold and Robertson (U. of Chicago), we have found that underweight anorectics have a subnormal and erratic release of vasopressin (AVP) to intravenous hypertonic saline. This abnormality persisted in patients studied 3 to 4 weeks after weight recovery, but disappeared in 5 of 7 patients studied after at least 6 months of weight recovery. Abnormalities in the osmoregulation of plasma AVP were almost always associated with

an absolute increase in the level of AVP in the cerebrospinal fluid (CSF) or a reversal of the normal CSF/plasma AVP ratio. These results indicate that most if not all patients with anorexia nervosa have abnormal levels of AVP in the plasma and CSF that are corrected very slowly with weight gain. Another intriguing finding is that anorectics, who are food restricters, appear to have higher concentrations of CSF 5-HIAA after probenecid than do anorectics who binge and vomit or use laxatives.

Further studies are underway in investigating norepinephrine (NE) metabolism and its effects on energy utilization in anorexia nervosa. In collaboration with Drs. Jimerson and Lake, we have found no change in mean levels of CSF, NE, MHPG, DHPG, or plasma MHPG between underweight anorectics and the same women within 4 weeks after weight recovery. Underweight anorectics eat a mean of 33 cal/kg/day when maintaining a stable weight. In the month after weight is recovered, anorectics require 52 cal/kg/day to maintain a stable weight. Long-term weight recovered subjects had significant decreases in CSF, NE, MHPG, DHPG, and plasma NE and MHPG compared to controls and a decrease in caloric intake to a mean of 27 cal/kg/day. Thus subjects able to maintain weight long term had a normalized caloric intake associated with a decrease in NE metabolism. Patients with anorexia nervosa who successfully maintain weight after refeeding may do so by changes in energy balance mediated by central and peripheral adrenergic systems.

The effect of oral clonidine (600 mg per day) on tremor amplitude measured with an accelerometer was studied in 6 patients with essential tremor. Three patients showed a marked decrease (60 - 70%) and 2 patients mild reduction (9 - 11%) in tremor amplitude accompanied by mild asymptomatic reductions in arterial blood pressure and heart rate, and mild transient side effects. A decrease in the availability of norepinephrine at α -receptors in the brain may account for the effect of clonidine on tremor amplitude. Clonidine, an α -agonist with a different mechanism of action than the widely-used β -blockers, represents a therapeutic alternative that may prove to be important in the treatment of patients unresponsive to propranolol and in patients with asthma or diabetes mellitus (Drs. Burns, Martin, Ebert).

In an effort to define the relationship between idiopathic Parkinson's disease and dementia, cognitive testing was performed in 6 Parkinsonian patients who had not previously received L-dopa or ergot compounds. Unlike patients with progressive dementias such as Alzheimer's disease, untreated Parkinsonian patients with mild-to-moderate motor impairment (Haehn and Yahr stage I - IV) are efficient at accessing previously acquired knowledge. They also can learn and remember information that can be processed "automatically" using operations requiring little cognitive capacity. However, these patients demonstrate distinctive impairments requiring effort-demanding cognitive processes (Drs. Burns, Weingartner).

A clinical study of the kinetics and metabolism of deuterium-labeled HVA in untreated Parkinson's disease patients was carried out in an effort to estimate the central (brain) contribution to total body HVA production, and to evaluate the relative value of CSF and plasma HVA levels and the urinary excretion rate of HVA as measure of brain dopamine metabolism. Twenty patients with Parkinsonism and 7 controls were studied. Preliminary analyses do not reveal any difference in the estimated total body production rate (about 200-250 μ g/hr) derived from the plasma kinetics of d₅-HVA. The CSF HVA concentration in

patients with stage I or II Parkinsonism (39 ± 17 ng/ml) was not different from controls (46 ± 15 ng/ml), demonstrating that motor signs precede the fall in CSF HVA levels. This is probably due to compensatory changes (increased turnover) in surviving dopaminergic neurons. Patients with stage III or IV Parkinsonism did have a lower CSF HVA concentration (9.9 ng/ml), and analyses of the HVA production rate in this group should provide an estimate of the contribution of the central nervous system to total body HVA production (Drs. Burns, Ebert).

An animal toxic model of Parkinson's disease in humans has been developed this year in collaboration with the Sections on Medicine, Histopharmacology and Analytical Biochemistry. Two young men who had developed persistent resting tremor, rigidity, akinesia and a flexed posture with low CSF levels of HVA ($6.5, 6.7$ ng/ml) following IV administration of an illicit drug preparation found to contain N-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (NMPTP) were studied. All of the clinical signs were reversed by the administration of L-dopa. Based upon the patients' pattern of use of the drug mixture, rhesus monkeys were given multiple doses (0.33 mg/kg) IV of NMPTP over 5 days and developed a similar neurological syndrome (akinesia, rigidity, flexed posture and postural tremor) which could be reversed by L-dopa. Within 7 days, the concentrations of HVA, MHPG, and 5-HIAA in ventricular CSF decreased to 37%, 38% and 60% of the baseline values, respectively. At 3 mo. MHPG and 5-HIAA had returned to 75% and 86% of baseline respectively, while HVA remained increased. A 90% decrease in the dopamine (DA) content of the putamen and caudate n. was found at 1 mo. Histofluorescence studies at 1 wk. and 1 mo. revealed tortuous, swollen, distorted axons of substantia nigra dopaminergic neurons whereas the locus coeruleus cells and axons remained normal in appearance. Light microscopy showed a marked loss of pigmented neurons in the pars compacta of the substantia nigra at 2 mo. NMPTP induces a permanent extrapyramidal state in both man and subhuman primates which faithfully reproduces the cardinal clinical features of human Parkinson's disease (PD) and is reversed by L-dopa. The pathological and biochemical changes produced by NMPTP are similar to the well established findings in PD. The NMPTP lesioned monkey represents a new and relatively complete model of human PD (Drs. Burns, Markey, Chieuh, Jacobowitz, Kopin, Ebert).

An attempted replication of reports of impaired central noradrenergic activity and memory enhancement with the α_2 -noradrenergic agonist clonidine hydrochloride in patients with Korsakoff's psychosis (KP) did not confirm the observations. There was no significant difference in lumbar cerebrospinal fluid (CSF) concentrations of norepinephrine, its major metabolite, 3-methoxy-4-hydroxyphenyl glycol (MHPG), nor the contribution of plasma to CSF free MHPG in 6 patients with clinically well characterized KP and 8 age-matched healthy normal volunteers. Likewise, CSF concentrations of the major metabolites of dopamine (homovanillic acid) and serotonin (5-hydroxyindoleacetic acid) were not different. In 7 patients with KP, clonidine treatment did not produce significant changes in measures of either semantic or episodic memory (Drs. Martin, Weingartner, Ebert).

During the above study, seven normotensive patients with KP treated with clonidine had reduced urinary excretion of NE metabolites: normetanephrine (NM), vanillylmandelic acid (VMA), and MHPG. The excretion of metanephrine (M) was not reduced significantly. Reduction in NM/(VMA + MHPG) indicates disproportionate lowering of the O-methylated metabolite of NE compared to its deaminated metabolites consistent with C inhibition of NE release (Drs. Martin, Kopin, Ebert).

SECTION ON CHILD PSYCHIATRY
Dr. Judith Rapoport, M.D., Chief

The Section conducts research on biological aspects of child psychiatry. Responses to pharmacologic agents as well as newer neuroradiological techniques are major tools for this research. Because research in child psychopathology is at an early stage of its development, a great deal of effort has gone into developing methodology for clinical research. This includes standardization of attentional tasks, of ward ratings, and activity monitoring.

For studies with obsessive-compulsive children, age appropriate rating scales were devised to measure rituals and intrusive thoughts. For hyperkinetic children the U.S. Diagnostic System (DSM III) is being compared with the European System (ICD 9) to evaluate the relative rarity with which this diagnosis is made in other countries.

Several collaborators from three other Institutes and from NIMH have participated in these child psychiatric studies. The NINCDS (Drs. Denckla, Fedio and Ludlow), NIA (Dr. Stanley Rapoport and staff), NICHD (Drs. Cornblath and Mukherje) and other branches within the NIMH: LPP, CP, CPP.

A variety of disorders are being studied as well as the response of normal children to dietary substances. These projects have in common the use of physiological responses correlated with behavioral change to understand the psychophysiology and pathophysiology in childhood behavior disturbance.

One major focus has been the hyperactive child, that is children with the diagnosis of Attention Deficit Disorder. A series of studies over the past seven years have addressed the hypothesis of a dopamine deficiency in this disorder. Results have supported nonadrenergic rather than dopaminergic mechanisms, although it seems probable that both systems are involved.

Newer neuroradiological techniques are being used increasingly. The studies with obsessive-compulsive children indicate CT scan differences from control groups (increased ventricular brain ratios) similar to that reported for some adult schizophrenics. A project with adults diagnosed as having Infantile Autism is examining both CT scans and PET scans in these patients and matched controls. Results indicate a possible diffusely increased metabolic rate in all regions examined.

More recent studies have examined dietary substances of considerable popular concern as possibly causing or augmenting behavior problems in children. A series of studies have examined the acute and subacute response of normal grade school children to caffeine (10 mg/kg). Preliminary results suggest that habitual caffeine intake predicts both baseline differences and response to caffeine. Children who self-selected high caffeine diets may possibly be selecting a beneficial calming agent. The behavioral response of hyperactive and normal children "thought to be sugar reactive" to glucose and sucrose have been studied. No disturbance of behavior was found even following relatively high dose challenges, but rather a slight but significant decrease in motor activity was observed two hours after the sugar challenge.

The response of grade school children to a single dose of ethanol was similar for offspring of alcoholics and controls, and blood and breath acetaldehyde did not differentiate the groups. However, ethanol tended to alter mood in a direction opposite to baseline state, such that children who were dysphoric reported greater pleasure from the experience than those who were not.

UNIT ON CLINICAL BIOCHEMISTRY
Mrs. Edna K. Gordon, Chief

The Unit on Clinical Biochemistry develops and applies new procedures for the quantitative analysis of biogenic amines and their metabolites in human body fluids and is involved in studies designed to evaluate the significance of changes in levels of turnover rates of these metabolites.

Although some believe that total urinary MHPG may reflect brain catecholamine metabolism, we have obtained good evidence which indicates that less than 30% of urinary MHPG is derived from brain. Methods using GC-MS have been developed for accurate assay of MHPG and its deuterated derivatives. Deuterated MHPG injected as a bolus or administered as a slow infusion over 3-4 hours is excreted in urine as a conjugate of MHPG or converted to VMA. Unconjugated plasma MHPG is a major transitional metabolite which accounts for about 2/3 of the total urinary catecholamine metabolites. About half of the MHPG in plasma is excreted as a conjugate whereas the remainder is converted to VMA and accounts for about half of the VMA excreted in the urine. Both conjugated MHPG and VMA are derived from sources independent of plasma MHPG, e.g., DHPG.

Plasma MHPG levels may, however, provide a useful index of cumulative sympathetic activity in the whole body. The levels of MHPG in plasma are significantly greater in depressed patients who fail to suppress plasma cortisol levels in response to dexamethasone than in patients who show normal suppression. There is an exchange of plasma and CSF MHPG so that plasma and CSF levels of free MHPG are highly correlated. CSF levels are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from the tumor of the adrenal medulla. Plasma and CSF behave like a two-compartment system with similar rate constants for entry into and exit from the CSF compartment. MHPG formed, but not metabolized, in the central nervous system maintains CSF levels. Deuterated MHPG has been shown to pass from plasma into monkey brain and CSF.

Methods for assay of urinary excretion of catecholamine metabolites have been developed and applied in a series of collaborative studies described in detail in other portions of this summary. Studies on Korsakoff's psychosis, orthostatic hypotension, anorexia nervosa, parkinson's disease, and anxiety-panic disorders are in progress.

ANNUAL REPORT OF THE CLINICAL NEUROSCIENCE BRANCH
National Institute of Mental Health
October 1, 1982 - September 30, 1983

Steven M. Paul, M. D., Chief

Introduction

This is the first annual report of the Clinical Neuroscience Branch, a recently established interdisciplinary group of neuroscientists. The Clinical Neuroscience Branch was formed during last year's reorganization by combining several existing sections and units of the Biological Psychiatry and Clinical Psychobiology Branches. The Branch consists of four sections: 1) The Section on Preclinical Studies (Dr. Steven M. Paul, Chief); 2) The Section on Molecular Pharmacology (Dr. Steven M. Paul, Acting Chief); 3) The Section on Brain Biochemistry (Dr. Candace B. Pert, Chief); and 4) The Section on Clinical Studies (Dr. David Pickar, Chief). Although each section has its own independent research program, the Branch has been designed and administered in such a way as to maximize the interactions and formal collaborations among and between basic and clinical neuroscientists. This concept while rather ambitious, has already resulted in a number of fruitful collaborations between primarily laboratory-based and clinically-based investigators. As Branch Chief, my overriding philosophy in administering the Branch has been to bring together a group of investigators into a creative environment, encourage them to follow their own scientific instincts, and to catalyze productive interactions among individuals with unique but complimentary skills. An investigator in our Branch could, for example, be found studying the functional significance of co-existing neurotransmitters (such as cholecystokinin and dopamine) using electrophysiological techniques (in studies of neuronal excitability in the substantia nigra), and simultaneously be examining the behavioral consequences of this co-existence vis-à-vis dopamine-mediated behaviors in rodents (i.e. modulation of stereotypy or motor activity). Moreover this same individual (or group of investigators) could actively be involved in clinical studies on the behavioral (and possible antipsychotic) effects of cholecystokinin (and related peptides) in schizophrenic patients.

There are a number of similar examples in which research on a particular problem is simultaneously conducted at the molecular and cellular level (i.e. membrane receptor, ion channel) as well as at the animal behavioral and even clinical level. I would like to emphasize that while such an interdisciplinary approach is common to most fields of medicine (especially in the other NIH Institutes) much of the research in mental health has historically been dichotomized into either "clinical" or "basic" disciplines. Many times neither type of investigator fully appreciates the problems and approaches of the other. Nonetheless, as our understanding of the central nervous system becomes increasingly more sophisticated, the application of this information to clinical problems will become increasingly feasible. The Clinical Neuroscience Branch by virtue of its members' interests and skills, should be in a good position to both contribute as well as apply these exciting new discoveries.

Over the past year there have been a few significant personnel changes in the Branch, as well as a subtle shifting of the Branch's resources into new research directions. The arrival of Dr. Jacqueline Crawley to head up the Unit on Behavioral Pharmacology has catalyzed a number of research projects (with investigators from all four sections) that have applied pharmacological, biochemical, and neurophysiological techniques to the study of animal behavior. Dr. Crawley has been joined by Dr. John Glowa, a Guest Worker, who has recently finished his postdoctoral fellowship with Dr. Peter Dews at Harvard University. Dr. Glowa has continued and extended our previous primate research on animal models of "anxiety" and "depression" by setting up and applying a number of operant conditioning paradigms (in rhesus and squirrel monkeys) that are highly sensitive to specific psychotropic agents. Again we anticipate combining these sophisticated behavioral techniques with the pharmacologic, neurochemical, neuroanatomical, and neurophysiologic expertise already present in the Branch. The ultimate goal is to "map out" the neuroanatomical and neurochemical circuitry involved in a number of primitive animal behaviors including experimental "anxiety" or "fear" and learned helplessness.

The departure of Dr. John Tallman, Chief of the Section on Molecular Pharmacology to become an Associate Professor of Psychiatry at Yale Medical School has left an unfortunate gap in the biochemical expertise of the Branch. Since members of both the Sections on Preclinical Studies and Brain Biochemistry have put increasing emphasis on the molecular and biochemical characterization of membrane proteins, including drug and neurotransmitter receptors and their associated ion channels, we have recruited a young immunochemist Dr. Richard Weber to help fill this void. Dr. Weber's interests and skills will undoubtedly facilitate and extend a variety of ongoing projects on the molecular and biochemical characterization of benzodiazepine and opiate receptors. Over the past year Dr. Weber has also collaborated extensively with Dr. Candace Pert on the development of a highly sensitive and specific immunoassay for detecting anti-brain antibodies in the sera of neuropsychiatric patients. This method will be applied shortly to a number of diseases of suspected autoimmune etiology. Dr. Weber's unit will also house the Branch's tissue culture facilities which when complete, will expand our capabilities at producing monoclonal antibodies (as specific membrane receptor probes) and allow us to carry out experiments using a variety of transformed neuronal and glial cell lines.

Over the past year the Clinical Research Unit under the leadership of Dr. David Pickar has gradually reoriented the direction of our clinical research program. Rather than restricting ourselves solely to the study of schizophrenia, we have expanded the scope of our investigations to include both the affective and anxiety disorders, as well as the Borderline Personality Syndrome. While presenting some administrative headaches, the clinical unit (4 East) now functions more in the spirit of a university-based psychiatric clinical research ward. The advantages of this diversified approach include a greater flexibility in asking productive and timely research questions without being constrained to a specific diagnostic category. As important however, is

our ability to compare biological findings across diagnostic boundaries, an endeavor that has become increasingly more important in psychiatric research over the past several years. The many examples of seemingly specific biological "markers" in psychiatric patients (positive dexamethasone suppression test in depression, increased ventricular brain ratios in schizophrenia) which subsequently fail to discriminate between diagnostic groups, attests for the need for careful and rigorous studies across (as well as within) diagnoses. Furthermore, such studies are more likely to reveal biological correlates of important symptoms or behavioral states that segregate independently of the larger syndrome (e.g. decreased CSF 5HIAA as a vulnerability marker for suicidal behavior). It is our opinion that this diversified approach will increase our effectiveness at studying meaningful biological factors as they relate to human psychopathology.

SECTION ON PRECLINICAL STUDIES

Steven M. Paul, M.D., Chief

The Section on Preclinical Studies continues to study the mechanism(s) of action of important psychotropic drugs. The principal drugs under investigation include the benzodiazepines, tricyclic antidepressants, and the psychomotor stimulants, amphetamine and methylphenidate. Many of these studies are carried out in collaboration with Dr. Phil Skolnick of the Laboratory of Bioorganic Chemistry, NIADDK. Over the past year, work on the benzodiazepine receptor has continued in a number of different areas. Purification of the benzodiazepine/GABA receptor complex using affinity chromatography has resulted in an approximately 1500-fold purification. This method is now being "scaled up" for preparing microgram quantities of the receptor for subsequent reconstitution experiments and for the production of monoclonal antibodies. Radiation inactivation experiments have revealed the molecular size of the various components of the benzodiazepine/GABA receptor complex *in situ*. Using frozen membrane preparations the target size analysis agrees well with the subunit molecular weight determined by photoaffinity labelling and SDS/PAGE chromatography. These experiments have also demonstrated that benzodiazepine receptor agonists, antagonists, and inverse agonists (active antagonists) all bind to the same molecular weight subunit, thus supporting the "domain hypothesis" as originally proposed by our laboratory.

Other studies on the benzodiazepines involve the biochemical and pharmacological characterization of the so-called "peripheral receptor" using the specific ligand 4-chlorodiazepam (Ro 5-4864). Over the past year we have demonstrated that Ro 5-4864 is a centrally active convulsant; and that the convulsant properties of Ro 5-4864 are not mediated through the previously established benzodiazepine receptor. A number of novel benzodiazepines have been tested and several have been shown to potently block the convulsions produced by Ro 5-4864. Electrophysiological studies carried out in the zona reticulata of the substantia nigra indicate potent excitatory actions of Ro 5-4864 that are not reversed by the selective benzodiazepine receptor antagonist Ro 15-1788 but are antagonized by GABA agonists such as muscimol. It has been postulated that Ro 5-4864 (4-chlorodiazepam) recognizes a novel benzodiazepine receptor and studies to characterize the exact site of action of this compound are in progress.

The Section on Preclinical Studies has also extended its previous work on the interaction of psychotropic drugs with presynaptic uptake sites. Specific "high affinity" and sodium dependent recognition sites for [^3H] imipramine and [^3H] desipramine have been characterized and shown to be structurally associated with the presynaptic uptake or transport "mechanisms" for serotonin and norepinephrine respectively. Platelet membranes have been used as an enriched tissue source for [^3H] imipramine binding sites and monoclonal antibodies have been raised against human platelet membranes. Several monoclonal antibodies have been shown by Dr. Itzchak Angel to selectively inhibit serotonin transport into platelets and these are now being fully characterized. Of these, several antibodies directed against the platelet membrane have also been shown to cross-react with neuronal membranes. Hopefully, these monoclonal antibodies will be helpful in elucidating the exact structural relationship between the tricyclic antidepressant binding site and the "transporter" itself.

Work on the previously described high affinity binding site(s) for [^3H](+)-amphetamine has also continued over the past year. Further characterization of [^3H](+)-amphetamine binding has revealed a very high localization to the CNS and particularly to the synaptosomal fraction of brain. Physiological changes in appetite (i.e. through imposed starvation and refeeding) results in significant changes (decreases and increases respectively) in specific [^3H](+)-amphetamine binding in the hypothalamus and brainstem of rats. In contrast, the genetically obese mouse (ob/ob) has been found to have a significant increase in the number (B_{max}) of [^3H](+)-amphetamine binding sites in various brain regions. In addition to the binding sites for [^3H](+)-amphetamine another psychomotor stimulant, [^3H]-methylphenidate, has been shown to bind to a saturable and stereospecific site which is again highly localized to the CNS (highest binding in the striatum). Displacement of specific [^3H] methylphenidate binding by various ritalinic acid esters and psychomotor stimulants was highly correlated to their behavioral potencies as motor stimulants, indicating that the [^3H]-methylphenidate recognition site may be a "receptor" for a variety of stimulant compounds. More recent work has localized [^3H]-methylphenidate binding sites to dopamine-containing terminals in the striatum. Studies to determine the relationship of these binding sites to the modulation of dopamine uptake and (or) release are in progress. Preliminary experiments indicate that sodium-dependent and sodium-independent binding sites for [^3H](+) methylphenidate exist in the CNS and that these sites differ in their pharmacological and biochemical characteristics.

The stimulation of inositol phospholipid metabolism in rat brain appears to be a receptor-mediated event that is sensitive to a number of neurotransmitter agonists including carbachol, serotonin, and phenylephrine. Recent work in the Section by Drs. Labarca and Janowsky have confirmed that the addition of lithium to brain slice preparations *in vitro* inhibits the breakdown of inositol phosphates to myo-inositol. Using this information Drs. Labarca and Janowsky have refined and developed a rapid method for determining phosphatidylinositol (PI) hydrolysis in hippocampal slice preparations. Preincubation of brain slices with [^3H] myo-inositol results in the radioactive labelling of PI. Subsequent hydrolysis of [^3H] PI to [^3H] inositol-1-phosphate can then be measured since the breakdown of the latter by inositol-1-phosphatase is inhibited in the presence of lithium. Using this method, the PI response to cholinergic, adrenergic, and serotonergic agonists has been characterized in hippocampal slices. The effects of chemical and physical lesions of the ascending noradrenergic pathway to the hippocampus on PI hydrolysis induced

by various agonists, have revealed previously unsuspected interrelationships between neurotransmitter systems. Further, the effects of acute and chronic administration of psychotropic drugs on neurotransmitter-induced PI hydrolysis have delineated a role for the ascending noradrenergic system in tonically regulating other neurotransmitter systems including the muscarinic-cholinergic and serotonergic (5HT₁) systems. The phosphatidylinositol hydrolysis assay appears to be an ideal model system for studying the interrelationships between neurotransmitters as well as the acute and chronic effects of psychotropic drugs on various neuroreceptors.

SECTION ON BRAIN BIOCHEMISTRY

Candace B. Pert, Ph.D., Chief

Dr. Candace Pert has continued her studies on the characterization and visualization of neurotransmitter and drug receptors in the CNS. Using a novel autoradiographic method developed with Dr. Miles Herkenham of the Laboratory of Neurophysiology, DIRP, Dr. Pert has characterized for the first time a number of neuropeptide receptors including those for Substance P, cholecystokinin, neurotensin, and various opiate peptides. The distribution of these receptors has been mapped out and many important correlations between neuroanatomic distribution and purported peptide function have been made. Substance P receptors, for example, share the same general locales with opiate receptors (such as various sensory areas and limbic structures) with opiate receptors, suggesting reciprocal modulation between these two peptides in the perception of "pain and pleasure". Cholecystokinin receptors have also been visualized for the first time and significant densities were observed in the cingulate cortex. Neurotensin receptors have been shown to be localized to cell bodies and terminals of many of the important dopamine pathways in the brain. These results help explain the many interrelationships between neurotensin and dopamine-mediated behaviors.

In addition to the autoradiographic methods for visualizing receptors Dr. Pert with Drs. McLean and Skirboll have developed a novel radioimmunochemical method for measuring a variety of antigens including neurotransmitters in the CNS. This method has been applied to studies on the opiate projections from cell bodies of the bed nucleus of the stria terminalis to the habenula. Obviously this method could have wide applicability to a variety of neurotransmitter systems and may ultimately prove to be fully quantitative; which will allow for measuring and visualizing regional changes in neurotransmitter levels.

Drs. Pert and Quirion have shown that the pattern of phencyclidine (angel dust) receptors is reminiscent of other neuropeptide receptors. Indeed, they have prepared peptide extracts of bovine hippocampus, (where angel dust receptors are highest) which have phencyclidine-like activity in binding assays and two behavioral assays. The active fraction(s) from brain is now being purified for subsequent characterization. Preliminary studies suggest that it is a peptide and substantial amounts are being prepared for subsequent purification and sequencing.

In related studies Dr. Pert, and collaborators in the Laboratory of Chemistry, NIADK, have developed an ¹⁸F-labelled high affinity opiate receptor ligand for positron emission tomography (PET) studies. The prototypic

compound represents a fluoride derivative of phenazocine which is similar to phenazocine in its affinity for opiate receptors in vitro. Studies are in progress to characterize the in vivo binding of this ¹⁸F derivative in rats and monkeys as a prelude to studies in man. Such a compound could prove invaluable for clinical studies on CNS opiate receptors.

The notion that schizophrenia has an important autoimmune component has been around for several decades but has not previously been subjected to analysis by the most sensitive and modern techniques. Dr. Pert and colleagues have developed a simple and sensitive assay for detecting antibodies directed against human brain from sera of schizophrenic patients and controls. They are now exploring the frequency of these antibodies in schizophrenics vs. controls and characterizing the molecular properties of the brain antigens involved; as well as their distribution by visualization in rodent and human brain. In collaboration with Dr. Richard Weber, over twenty experiments have been performed for the purpose of optimizing the conditions of the antibody detection assay. In an early blind experiment, serum samples from six of the Clinical Center 4-East ward acute schizophrenics and controls were examined. The two highest numbers (i.e. highest titres) in the assay belonged to the two most psychotic patients. Utilizing the sera from these two patients and controls Drs. Pert and Weber have worked on optimization of the assay. The assay now appears to be highly sensitive and to repeatedly demonstrate differences in these sera and that of another patient whose serum was recently screened by Dr. Lynn DeLisi. The latter patient's serum level of the active fraction appears to be elevated even after repeated blood sampling over a period of one year. Dr. Pert and her colleagues, are simultaneously refining and developing their assay (using an ELISA method) as well as screening patients and controls for the presence of anti-brain antibodies. This new radioimmunoassay method, for rapidly screening sera for the presence of anti-brain antibodies, will ultimately be applied to a number of other neuropsychiatric conditions including childhood autism and Alzheimer's disease.

Previous work by Dr. Pert has demonstrated that small cell carcinoma of the lung is characterized by the presence of the neuropeptide bombesin; which is also excreted into the blood of patients during the terminal stages of their illness. Dr. Pert's studies on small cell carcinoma of the lung over the past year indicate that the true origin of the disease may be in the hematopoietic system, in cells arising from the bone marrow. Using monoclonal antibodies that characterize macrophage cell surface antigens she has shown significant cross-reactivity to small cells. Furthermore, it appears that bombesin is chemotactic for hematopoietic cells which become blood elements like macrophages. Thus, Dr. Pert has postulated that small cell carcinoma of the lung may represent a primitive or dedifferentiated macrophage. Further studies on the immunochemical characterization of cell surface markers for macrophages on small cell carcinoma cells are in progress.

SECTION ON MOLECULAR PHARMACOLOGY

Steven M. Paul, M.D., Acting Chief

The Section on Molecular Pharmacology at present consists of two functional units, the Unit on Neurophysiology and Histochemistry (Drs. Lana Skirboll and Daniel Hommer) and the Unit on Behavioral Pharmacology (Dr. Jacqueline Crawley).

Drs. Skirboll and Hommer have continued their studies on characterizing coexisting neurotransmitter systems in both the central and peripheral nervous systems. Using immunocytochemical techniques combined with retrograde tracing with fluorescent dyes, they have extended their studies on a coexisting cholecystikinin (CCK), Substance P (SP) pathway in the brain and spinal cord. Examination of supraspinal projections in the rat have shown that SP neurons in the raphe magnus project to the ventral horn of the spinal cord. In addition, an enkephalin-immunoreactive system has also been shown to give rise to descending axons from cell bodies located dorso-lateral to the pyramidal tracts. Other projections to the spinal cord include somatostatin-immunoreactive cell bodies from the intercommissuralis and the A₁/C₁ cell group as well as a coexisting serotonin (5HT)-thyrotropin stimulating hormone (TRH)-SP pathway from cell bodies of the medullary raphe to the ventral horn. Using retrograde tracing, Dr. Skirboll and her collaborators have also provided strong evidence that neurons from the paraventricular nucleus of the hypothalamus (PVN) project to the A₁/C₁ group and contain vasopressin, neurotensin, CCK, and TRH. In an extension of last year's work Drs. Skirboll and Hommer have demonstrated a tri-transmitter system (5HT-SP-TRH) which projects to the ventral horn of the spinal cord and to the phrenic motor nucleus in the cat. Studies are now in progress to characterize the significance of these coexisting neurotransmitters in at least some of these systems, using neurophysiological techniques.

Over the past year Dr. Skirboll in collaboration with Drs. Stafford McLean and Candace Pert have developed a novel radioimmunohistochemical technique for visualizing (and semi-quantifying) antigen-antibody complexes in cryostat sections of brain. This technique has been applied to examining the distribution of SP and enkephalin-containing neurons in various neural pathways. The use of an ¹²⁵I-labelled secondary antibody allows for a rapid development time and good autoradiographs can be prepared in just a few days.

Using extracellular single unit recording techniques Drs. Hommer and Skirboll have continued their work on the interaction of CCK and dopamine in the substantia nigra. The major finding over the past year has been that systemically administered CCK produces DA autoreceptor "supersensitivity". Iontophoretically applied CCK also has been shown to potentiate the inhibitory action of iontophoretically applied DA. These findings provide a possible mechanism for many of CCK's reported behavioral (cf Dr. Crawley's work below) and clinical effects and represent some of the first studies to establish the possible functional significance between two coexisting neurotransmitters. In related studies Drs. Hommer and Skirboll have also demonstrated neurons in the olfactory tubercle that are excited by systemically administered CCK. Moreover, these neurons are inhibited by the DA agonist apomorphine; whereas neurons that are not excited by CCK are also not inhibited by apomorphine. Thus, CCK selectively acts on neurons which are sensitive to DA. Other work has attempted to elucidate the exact site of action of parenterally administered peptides like CCK. Is the excitation of DA neurons produced by systemically administered CCK a result of the peptides direct effect on these neurons. To answer this question rats were prepared with various lesions (in collaboration with Dr. M. Palkovitz) including complete vagotomy (acute and chronic), unilateral lesions of the nucleus tractus solitarius, and spinal cord (C₁) transection, just prior to recording. Since none of these lesions altered CCK's ability to excite DA neurons it appears that this effect (in contrast to some of the behavioral actions of CCK) is not mediated via peripheral mechanisms.

In other studies in collaboration with members of the Section on Preclinical Studies, Dr. Hommer has studied the neurophysiological actions of β -carboline ester ligands of the benzodiazepine receptor. Compounds such as β -CCE potently excite neurons in the zona reticulata of the substantia nigra and their effects were found to be reversed by selective benzodiazepine receptor antagonists such as Ro 15-1788. In contrast Ro 5-4864 (the "peripheral" benzodiazepine receptor ligand) also increased the activity of these neurons but this effect was shown not to be reversed by Ro 15-1788. However, muscimol potently inhibited Ro 5-4864-induced excitation suggesting a GABA inhibitory action of Ro 5-4864.

Dr. Jacqueline Crawley who joined the Branch this past year to set up a Unit on Behavioral Pharmacology, has continued her work on the behavioral actions of neuropeptides. A major emphasis has been placed on the neuromodulatory role of CCK on dopamine-mediated behaviors. CCK₈-sulfate when injected directly into the nucleus accumbens of the rat has been shown to potentiate the behavioral actions of the DA agonist apomorphine. CCK alone has minimal behavioral actions but appears to facilitate the actions of DA agonists. This behavioral "sensitization" by CCK is similar to that observed in the electrophysiological experiments of Drs. Hommer and Skirboll. Dr. Crawley has also demonstrated that peripherally administered CCK mimicks a number of "behaviors" associated with the state of "satiety". Reductions in exploratory behaviors and social interaction are the most prominent. The behavioral syndrome produced by CCK has also been shown to be significantly different from that produced by other "gut" peptides, including bombesin. Further, the reduced exploration produced by CCK was found to be due to accelerated habituation to novelty rather than to a nonspecific sedating effect of the peptide. Previous studies by Dr. Crawley have shown that the behavioral effects of systemic CCK are abolished by vagotomy. This observation suggests a novel pathway for mediation of the behavioral actions of gastrointestinal peptides. More recent studies using CCK peptide fragments indicate that the behavioral actions of CCK are mediated by different receptors than those that mediate pancreatic amylase secretion.

Dr. Crawley has also continued her studies on the behavioral pharmacology of anxiety. Using several benzodiazepine receptor antagonists she has further characterized the behavioral syndrome produced by these compounds in rhesus monkeys. Of these compounds the "active" benzodiazepine receptor antagonist (inverse agonist), 3-carboethoxy- β -carboline (β -CCE) produced an extreme behavioral response in chair-adapted rhesus monkeys, ranging from mild to severe "anxiety" symptoms. A newly-designed behavioral rating system, using 22 parameters specific for anxiety in rhesus monkeys, was scored once a minute for 30 minutes after β -CCE administration. Behavioral responses correlated well with increases in blood pressure, heart rate, plasma cortisol, ACTH, and β -endorphin. Effective pharmacological blockers of the β -CCE response were diazepam, clonidine (10 μ g/kg presynaptic dose), cyproheptadine, and morphine. Pretreatment with other drugs including the β -adrenoreceptor blocker propranolol produced no significant blockade. These findings suggest that α -2 noradrenergic serotonin and opiate receptors may be integral to the neural network which is activated by β -CCE administration.

A series of β -carbolines and other benzodiazepine receptor antagonists was similarly administered to mice in the light-dark exploratory model for anxiety previously developed by Dr. Crawley. None of the antagonists tested had any intrinsic anxiogenic activity in this system, although all blocked the anxiolytic actions of diazepam. These results suggest that benzodiazepine receptor antagonists may be active anxiogenics in primates, less active in rats, and inactive in mice. Metabolism of β -CCE may be responsible for this difference since a ten-fold monkey/rat differential of in vivo half-life of β -CCE has been documented in our laboratory.

Dr. Crawley has also extended her preliminary studies on a possible hamster separation model of "depression". A breeding colony of dwarf hamsters (Phodopus sungorus) has now been established in collaboration with Dr. Paul McLean at NIMH in Poolesville. Non-littermate males and females are being paired, separated, and repaired. Behavioral observations of the paired and separated states have been quantitated. Separated hamsters show increases in body weight, are less exploratory in a challenge novel environment, and less socially novel interactive with an unfamiliar animal of the opposite sex. Males show greater changes during separation than females indicating that this model may represent an animal analogue of human bereavement, since males are more highly susceptible to psychological and physiological disorders following loss of their spouse.

Preliminary HPLC assays of monoamines and metabolites from brain regions of paired and separated hamsters have found large decreases in serotonin turnover during the separated phase. The first drug trial using daily injection of imipramine during the separated period produced a reversal of approximately half of the affected parameters. This model shows promise as a novel rodent paradigm to investigate brain neurochemical and receptor changes during a behaviorally-induced "depressive" state.

Dr. Marian S. Kafka continues her studies on the characterization of α -adrenergic and prostaglandin receptors in human blood elements and particularly in various neuropsychiatric disorders. She has extended her previous findings of alterations in platelet α_2 -adrenergic receptor density in schizophrenia and depressed patients and has worked out novel techniques for studying the "coupling" of receptor and "second messenger" systems (adenylate cyclase) in platelets. Major findings over the past year include a study of the effects of chronic clorgyline administration to depressed patients on the number of platelet α_2 receptors and the prostaglandin E_1 (PGE_1)-stimulated cyclic AMP (cAMP) response in these same individuals. Despite changes in basal cAMP production and decreased circulating plasma NE levels, no changes in α_2 -adrenergic receptor density or PGE_1 -stimulated adenylate cyclase was observed. In related experiments Dr. Kafka has provided evidence for a subsensitivity or "blunting" of the norepinephrine-inhibited PGE_1 -stimulated adenylate cyclase in platelets from depressed patients compared to schizophrenics and healthy controls. This finding coupled with previous work on the labelling of platelet α_2 adrenergic receptors with [^3H] DHE, suggest a possible abnormality in the "catalytic unit" mediating norepinephrine inhibition of PGE_1 -stimulated adenylate cyclase in depression. New methods are being developed in order to extend this finding to more patient populations and on a routine basis.

Dr. Kafka and her collaborators have also extended their findings on circadian rhythms in neurotransmitter receptors in relatively discrete brain regions. Dr. Kafka has demonstrated that there are circadian rhythms in receptors not only in the large brain regions previously measured ("forebrain," i.e., the brain rostral to the cerebellum but without the striata), but in smaller regions, e.g., the cerebral cortex, hypothalamus, and the pons-medulla. In addition, she and her coworkers have found a circadian rhythm in an intracellular biochemical response to α_1 - and β -adrenergic receptor occupancy, viz. the norepinephrine (NE)-stimulated cAMP production in cerebral cortical slices. The rhythms in cAMP production resemble the rhythms in α_1 - and β -receptors supporting the physiological significance of the previously described circadian changes in receptors.

SECTION ON CLINICAL STUDIES

David Pickar, M.D., Chief

The Section on Clinical Studies which includes the 4-East Clinical Research Unit has just completed its first full year under the leadership of Dr. David Pickar. During the past year Dr. Pickar has reoriented several of the existing projects on schizophrenia research and initiated a number of new protocols in the area of affective and anxiety disorders. Thus, the clinical research ward now has the flexibility to address a number of research questions simultaneously and across diagnostic boundaries. Studies on the neurobiology of schizophrenia still comprise a major focus of the clinical studies in the Branch. Dr. Pickar and his colleagues are pursuing three principal areas of investigation including: 1) the neuropharmacology and neurochemistry of neuroleptic response; 2) the neuropsychiatric aspects of schizophrenia, and 3) the clinical and biological correlates of depressive and "negative" symptoms in schizophrenic patients.

One of the few consistent and highly reproducible findings in the area of schizophrenia research is the favorable response to antipsychotic agents seen in most patients. These drugs, collectively called neuroleptics, are believed to work by selectively antagonizing the actions of dopamine at non-adenylate cyclase linked DA receptors in the mesolimbic DA system. Despite the extensive indirect evidence (primarily derived from animal studies) for the involvement of dopamine in the antipsychotic actions of neuroleptics, very little support for this hypothesis can be found from studies conducted in schizophrenic patients themselves. For this reason, members of the Section on Clinical Studies are systematically examining the biochemical correlates of neuroleptic response by correlating behavioral changes associated with neuroleptic administration and discontinuation with longitudinal measures of plasma and CSF monoamines and their metabolites. Preliminary results have demonstrated significant changes in plasma HVA (a major dopamine metabolite) during chronic administration of neuroleptics. To date significant decreases in both the absolute plasma levels of HVA as well as the day to day variation in HVA levels seems to correlate with favorable antipsychotic effect. Studies are in progress to establish whether the neuroleptic-induced changes in plasma HVA are specific, or whether a variety of other neurotransmitter metabolites are also similarly effected. Nevertheless these studies are among the first to show correlations between neuroleptic-induced alterations in plasma HVA and clinical response and thus support the "dopamine hypothesis" of neuroleptic action.

The advent of new noninvasive techniques for studying the morphology of human brain in vivo has rekindled interest in possible neuropathological changes in schizophrenia. The most widely used technique-computerized axial tomography (CAT) has revealed increases in the ventricular brain ratio (VBR) as well as other signs of brain atrophy (e.g. sulci and third ventricular dilatation) in some schizophrenic patients when compared to healthy controls. Moreover, several authors have demonstrated a relationship between treatment responsivity (or lack thereof) and increased VBR. Drs. Pickar and Boronow using planimetric methods to "blindly" analyze CAT scans have measured the sizes of both the lateral and third ventricles in a substantial series of schizophrenic patients and medical controls. No changes in the dimensions of the lateral ventricle (or sulci width) were observed, however, a significant enlargement in the diameter of the third ventricle was observed in the schizophrenic patients compared to medical (but non-psychiatric) controls. There were also no significant correlations between ventricular size (VBR) and any clinical or biochemical variable examined. These results have highlighted the heterogeneity in the various populations of schizophrenic patients studied from one center to another and suggest that in younger patients, brain atrophy per se is not a very common correlate to the illness. Nevertheless, future studies on neuropathologic alterations in brain tissue surrounding the third ventricle may be informative and are currently in progress.

In related studies Drs. Pickar and Roy are examining the relationship between neuroleptic response, negative symptoms (i.e. poverty of speech, withdrawal, etc.), and affective symptoms in schizophrenic patients. Reported biological abnormalities in depression (e.g. DST nonsuppression, blunted TSH response to TRH) are now being systematically examined for in schizophrenic patients. To date, a significant percentage of schizophrenic patients have been found to have abnormal DST results and an attempt is now under way to relate this finding to either "depressive" or "negative" symptomatology.

Drs. Roy and Pickar have also initiated a rather large scale program to evaluate the true utility and diagnostic value of a number of purported biological "tests" for depression. These "tests" include: neuroendocrine (DST, TRH-TSH, plasma ACTH, β -endorphin, cortisol, etc.), neurotransmitter metabolite (plasma MHPG, HVA; CSF MHPG, HVA, 5HIAA, urinary metabolites), and membrane marker (platelet [^3H] imipramine binding; serotonin uptake, α_2 -adrenoreceptors, inhibition of PGE $_1$ -stimulated adenylate cyclase, etc.) measures. In addition to the various biological tests the patients' affective symptoms as well as various personality traits are carefully and extensively rated using a battery of rating scales and personality inventories. Life events during the antecedent 6 month period are recorded using Paykel's method and premorbid marital and social support assessments are also made. Patients are recruited for this diagnostic study only and no treatment protocols are attempted. In this way the number of patients made available for this protocol (as well as for other acute biological ongoing studies in depression) is significantly greater than would otherwise be possible. To date, approximately 50 depressed patients have been studied in only an 8 month period. This group includes 25 recurrent affective disorder patients, 9 dysthymic disorder patients, and 12 patients with a major depressive episode. Thus, both the sensitivity and specificity of the observed biological abnormalities will be examined over a wide spectrum of affectively ill patients. At present, data from the first 50 patients and controls is being analyzed.

Previous studies using the opiate receptor antagonist, naloxone, have shown little or inconsistent behavioral effects in normal humans. In order to assess the notion that previous doses were insufficient to yield a complete opiate receptor blockade, normal volunteers were administered increasing doses of naloxone (0.3-4.0 mg/kg) in a single-blind study. Drs. Pickar and Cohen have observed significant dose-dependent behavioral, hormonal (cortisol and growth hormone) and physiological effects associated with increasing doses of naloxone. With high doses of naloxone, healthy volunteers experienced increasing dysphoria, deterioration of performance on memory testing, increased systolic blood pressure and respiratory rate. In a separate double-blind study using a 2 mg/kg dose of naloxone administered to 7 normal volunteers, naloxone significantly reduced total food intake from preselected prepared trays served at 2.75 and 7.75 hours following drug administration. These data are consistent with animal studies demonstrating opiate receptor modulation of food intake and suggest further studies for the use of naloxone in treating various eating disorders.

Dr. Pickar's group has also measured plasma β -endorphin in patients with major depressive and minor depressive disorder and normal controls. Minor depressives had significantly less plasma β -endorphin than did patients with major depression or controls. In contrast, patients with major depressive disorder demonstrated predictable elevations in plasma cortisol compared to the other study groups. In major depressives, but not other groups, plasma β -endorphin was directly correlated with plasma cortisol. This finding complements earlier work from our group suggesting a link between the HPA axis and the endogenous opioid system in major depression.

Studies on the relationship between the endogenous opioid system, HPA axis, and obesity have been carried out in collaboration with Fairfax Hospital. Morning plasma cortisol and β -endorphin levels were found to be no different in obese patients, prior to diet treatment, than normal weight relatives. Plasma cortisol levels were significantly correlated in obese patients with self-ratings of depression. During the course of a 400 calorie per day modified protein fast significant decreases in levels of plasma cortisol but unchanging levels of plasma β -endorphin were observed.

In preliminary experiments Dr. Pickar and associates have administered intrathecal β -endorphin to terminal cancer patients for the control of severe pain. In both patients long-lasting analgesia was produced and one of the patients experienced a profound behavioral syndrome characterized by hypomania, psychosis, and confusion. Further work is in progress to examine the intrathecal route of administering opiate peptides for the alleviation of pain as well as unraveling the behavioral effects of such peptides.

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Mortimer Mishkin, Ph.D., Chief

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Two cortical visual pathways

Converging evidence from our earlier neurobehavioral, physiological, and anatomical studies indicate that the striate cortex in the monkey is the source of two multisynaptic corticocortical pathways. One courses ventrally interconnecting the striate, prestriate, and inferior temporal areas and is crucial for the visual identification of objects; links between this occipitotemporal pathway and limbic structures in the temporal lobe as well as ventral portions of the frontal lobe appear to make possible the cognitive association of visual objects with other events, such as emotions and motor acts. The other visual pathway runs dorsally, interconnecting the striate, prestriate, and inferior parietal areas and is critical instead for the visual location of objects; links between this occipitoparietal pathway and both dorsal limbic and dorsal frontal cortex enable the cognitive construction of spatial maps, as well as the visual guidance of motor acts that were initially triggered by activity in the ventral pathway. In contrast to the occipitotemporal pathway, which remains modality specific throughout its course, the later stations in the occipitoparietal pathway receive convergent input from other modalities and so constitute polysensory areas.

We have now demonstrated the entire cortical visual system at work by use of the [^{14}C]2-deoxyglucose method developed at NIMH by Sokoloff and his group in the Laboratory of Cerebral Metabolism. In collaboration with that laboratory we have found by comparing a blinded and a seeing hemisphere in the same monkey that all tissue related to vision can be clearly identified on the basis of differential hemispheric uptake of the 2-deoxyglucose during visual stimulation. The studies were carried out in awake monkeys presented with visual patterns either in a rotating drum or in a discrimination apparatus. In the latter case, the animal performed the discrimination for water reward using the hand opposite the blind hemisphere. In both situations reduced glucose utilization in the blind as compared with the seeing hemisphere was found cortically throughout the entire expanse of striate and prestriate cortex (areas OC, OB, and OA), inferior temporal cortex as far forward as the temporal pole (areas TEO and TE), and inferior prefrontal cortex (area FD_v). Dorsally, the area of reduced LCGU included the posterior part of the inferior parietal lobule (area PG) and prearcuate cortex (area FD_Δ). These results, which are in remarkably close agreement with our neurobehaviorally derived model of the two cortical visual pathways, have allowed us to delineate the exact limits of the entire system, the visual/nonvisual borders having turned out to be sharp and highly consistent among animals. These

borders outlined more cortical tissue than is generally recognized as being related to vision, including the fundus and part of the upper bank of the intraparietal sulcus, the posterior part of the parahippocampal gyrus, and the lateral bank of the rhinal sulcus. Also, the borders appeared reliably at zones of architectonic transition, lending new functional validity to cortical architectonics. Finally, tissue related to vision could be traced subcortically into parts of the limbic and striatal systems, findings that are relevant to later sections of this review.

Organization of prestriate cortex

To trace the flow of visual information through the large expanse of visual cortex revealed by the metabolic studies, we have undertaken a series of mapping studies using axonal transport and axonal degeneration techniques. Our goal in these anatomical investigations has been to identify the multiple visual areas within the prestriate cortex, explore their organization, and map their projections forward into both the temporal and parietal lobes. In the course of these studies, we have developed a myeloarchitectural stain that clearly distinguishes among the multiple prestriate areas for the first time.

Our findings thus far confirm the prediction that the striate cortex is the source of two major cortical projection systems. The first system begins with the striate projection to the second visual area, V2, which in turn projects to areas V3 and V4. These three prestriate areas are arranged in adjacent "belts" that nearly surround the striate cortex, and, like striate cortex, each belt contains a complete representation of the visual field. Area V2 corresponds to prestriate area OB, while V3 and V4 are both contained within prestriate area OA, exclusive of its dorsal part. Area V4 in turn projects to both areas TEO and TE in the inferior temporal cortex.

The second major system begins with both striate and V2 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within dorsolateral OA. Area MT in turn projects to four additional areas in the upper superior temporal and intraparietal sulci. Although the total extents of these four areas are not yet completely established, the more anterior one in the intraparietal sulcus clearly falls within area PG. Thus, one major system of projections out of striate cortex is directed ventrally into the temporal lobe, while a second is directed dorsally into the parietal lobe. Furthermore, the divergence between these two systems appears to begin almost immediately after striate cortex, i.e. in its initial projections.

While continuing to map these two corticocortical systems, we have also begun to investigate their subcortical connections. Our findings indicate that, like striate cortex, both areas MT and V4 project to the inferior and lateral pulvinar, superior colliculus, and reticular nucleus of the thalamus, but unlike striate, both prestriate areas project in addition to the putamen, caudate, claustrum, and pontine grey. The considerable overlap in the subcortical projections from MT and V4 suggest that the contribution of each

area to subcortical processing lies not in a unique set of subcortical projections but rather in the unique information each supplies. As will be seen in the next section, this information concerns direction of stimulus motion for MT and stimulus form and color for V4.

The two multisynaptic projection systems that we have traced constitute part of the anatomical substrate for object vision and spatial vision, respectively. In addition, our anatomical results provide a partial solution to a puzzle that has persisted in the literature for decades, namely, why extensive bilateral removals of prestriate cortex in monkeys have repeatedly failed to yield the expected losses in either object or spatial vision. If prestriate cortex constitutes an essential relay in both a striate-temporal and a striate-parietal pathway, then damage to this relay should yield effects at least as severe as damage to both its target areas. Yet, such dramatic effects have not been found. The reason appears to be that no prestriate lesion to date has produced a total visual disconnection of the temporal and parietal lobes, since all removals have spared varying extents of prestriate tissue that could continue to relay visual information. Comparison with our anatomical maps indicates that the portions of prestriate cortex that have consistently escaped damage are those parts of both the belt areas and the MT-related areas that represent the peripheral visual fields. Thus, just as we and others have found with sparing of peripheral field representations in striate cortex, such sparing in prestriate cortex will protect both object and spatial vision from serious losses.

Although the basic reason that prestriate ablations have failed to yield the anticipated visual deficits has thus been clarified, the problem created by the negative findings is still not completely solved. For, unlike animals with lateral striate lesions, in which the spared peripheral field representations constitute fully half of the total extent of striate cortex, animals with some of the more massive prestriate lesions that have been reported in the literature had spared peripheral field representations that constitute less than one-third of the total prestriate cortex; and even this is undoubtedly an overestimate, since some of the tissue that survived was presumably disconnected from its visual sources or targets by invasion of the white matter in the preoccipital region. Finally, the prestriate tissue that did remain functional was necessarily divided among the many prestriate subdivisions that have now been discovered, all of which need not have represented precisely the same portion of the peripheral visual field. The question remains, therefore, how such prestriate remnants serving peripheral vision can continue to receive, process, and transmit sufficient information to allow the later stations in the two visual pathways to perform their highly complex visual functions at only slightly reduced levels. Only when the details of visual processing through the two cortical pathways are fully understood will this prestriate-lesion paradox be fully resolved. To gain some insights into visual processing we have undertaken a number of electrophysiological studies.

Prestriate cortex and the analysis of motion and figure-ground relationships

Now that we know the location, topography, and connections of many of the prestriate subdivisions we can compare their neuronal properties and trace the transformation of visual information through them. So far, we have recorded from two prestriate areas, V4 and MT, and have found striking evidence for functional specialization in both. In area MT, it has been known that neurons are not sensitive to the form or color of a stimulus but are highly sensitive to the direction of stimulus motion. We have discovered an elaborate columnar system for direction of motion in MT, comparable to the columnar system for orientation discovered by Hubel and Wiesel in striate cortex. Within a vertical column in MT, all cells respond to the same direction of motion. Moving across the columns, the preferred direction of motion changes systematically, resulting in a clock-like representation of direction. The sensitivity of MT neurons to where a stimulus is moving, rather than to what the stimulus is, is consistent with the strong anatomical connections of MT with areas of the posterior parietal cortex, which is necessary for processing the spatial relationships among objects. By contrast, in area V4, neurons are not sensitive to motion but are highly sensitive to object contours and color. In addition to sensitivity to the length, width, orientation, color, and spatial frequency of a stimulus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. This ability to respond to differences is due to a unique receptive field structure in V4: a small excitatory receptive field surrounded by a large, silent suppressive zone with properties antagonistic to those of the excitatory field. Thus, V4 neurons may play a role in separating figure from ground, a fundamental task in visual perception. Like the properties of MT, those of V4 are also consistent with its anatomical connections, since V4 provides the crucial link in the relay of information along the ventral pathway for object vision into the inferior temporal cortex.

Inferior temporal cortex and the analysis of shape

The discrimination of complex visual stimuli normally depends on a variety of features, such as brightness, size, texture, and color, but probably the most salient visual feature is shape. In previous studies of the properties of inferior temporal neurons, we found that most neurons were in fact highly sensitive to the shapes of objects. Since very few of these neurons are sensitive to the local features of objects, such as the location and orientation of single edges, they presumably code some global measure of shape. The problem is to identify that measure.

It is well known from the computer pattern-recognition literature that a particularly potent global measure of shape is the Fourier descriptor. The Fourier descriptors are a basic set of shape functions that can be used to generate any shape; likewise, any object can be decomposed into a unique set of these shape descriptors. The descriptors thus provide a powerful method for classifying shapes. Is it possible that inferior temporal neurons use such a method?

To answer this question, we have been studying responses of inferior temporal neurons to a set of Fourier descriptors. So far, over half the neurons tested have been sharply tuned to the set, that is, each of the tuned neurons responds best to a particular descriptor in the set. Furthermore, most of the tuned neurons exhibit size constancy in that they respond best to a particular descriptor regardless of size. These results strongly support a Fourier descriptor-like model of shape analysis. We are continuing to investigate other models of shape analysis, however, especially spatial frequency decomposition, to determine if the Fourier descriptor model is in fact the best explanation for our results.

Whereas the foregoing single-unit studies are being carried out on lightly anesthetized and immobilized preparations, others are being performed on the awake, behaving monkey while the monkey's fixation is closely monitored by a magnetic search-coil system. In our initial experiments with this technique, we found that active fixation of a tiny spot exerted an unexpectedly strong suppressive effect on neuronal responses to visual probe stimuli. If the fixation spot was turned off briefly while eye position was maintained, the response of the neuron to the probe stimuli increased dramatically. The improvement of neuronal responses to the probe stimuli when the fixation spot was absent could not have been due to a shift in attention to the spatial location of the probe stimuli, for when such a shift in spatial attention was explicitly required (by requiring the animal to respond to a slight dimming of the probe rather than of the fixation spot), the neuronal responses to the probe stimuli were weakened even more. In short, both the physical presence of the fixation spot and spatial attention to the probe were suppressive influences. This suppressive effect of attention to the probe stimuli, however, was obtained on tasks in which the shape of the probe stimuli was irrelevant for correct performance. In subsequent studies we made the shape relevant, by requiring the animal to discriminate between differently shaped probes, and under these conditions the strength of the neuronal responses to the probe stimuli was dramatically enhanced. Besides providing additional evidence that inferior temporal neurons are engaged in processing stimulus shape, these experiments show for the first time that the responses of inferior temporal neurons are markedly altered depending on which stimulus feature, location or shape, the animal is attending to. The results suggest the interesting possibility that the dorsal cortical visual pathway, specialized for spatial vision, is responsible for the suppressive influence that attention to spatial location exerts on the activity of inferior temporal neurons. Study of the functional interaction between the two cortical visual pathways at the neuronal level should provide valuable new insights into the mechanisms underlying selective visual attention.

[The anatomical experiments described earlier also bear upon this issue of selective attention. Materials from the anatomical experiments were used to investigate not only the cortical projections from striate cortex but also its subcortical projections to the pulvinar nucleus in the thalamus. In the corticocortical studies we had found that striate efferents to the prestriate area V2 (or OB) are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to V2. In the study

on striate efferents to the pulvinar, we found that once again there was a precise visuotopic organization, indicating that there are two sources of striate input to V2 in perfect register: one, direct, i.e. corticocortical; and the other, indirect, via the pulvinar. The latter system of inputs to V2 provides a possible mechanism by which signals (instructions, sets, etc.) from outside the visual system operating through the pulvinar could acquire visual field specification and thereby direct the animal's attention to a particular part of the visual field. Future anatomical studies will investigate these possible sources of nonvisual input to the pulvinar.]

As part of the experiments carried out in awake, trained rhesus monkeys, a new and powerful technique has been developed to study how single inferior temporal neurons encode visual patterns. The technique makes use of a set of visual stimuli that can be used to reconstruct any visual image. The entire set, consisting of 64 black and white stimuli comprising a complete, orthogonal 2-dimensional set based on Walsh functions, is presented one at a time at a controlled retinal location. The neuronal response to each of the 64 stimuli is then transformed into a continuous probability function of spike occurrence. Inspection of the 64 responses for any given neuron shows that several of the responses differ from the others in waveform. To quantify the differences we decompose the waveforms into their principal components using a Karhunen-Loeve transform. One desirable feature of this transform is that it represents the 64 waveforms with the fewest possible linear coefficients, i.e., no fewer linear parameters can represent the waveforms so accurately. The analysis shows that three to five components of the transform are usually needed to fully represent the differences in neuronal responses to the 64 patterns, a result which gives us strong reason to believe that visual features are encoded not simply in mean firing rate but in the temporal spike train of the neuronal response. Moreover, similar experiments conducted on single neurons in striate cortex have shown that two to three principal components are needed to represent the waveforms of these neurons to the set of Walsh patterns. Since neurons of both the first and last visual cortical stations (striate cortex and inferior temporal cortex, respectively) seem to modulate their temporal waveforms in response to different visual features, such modulation is likely to be a general property of the entire cortical system serving object vision. By using different combinations of these temporal waveforms, single units have the potential for conveying very specific information about complex patterns from one station in the visual system to the next. These quantitative methods not only open up an entirely new dimension for the analysis of single-unit activity but also should allow us to investigate critical issues that have been difficult to approach until now. One such issue we intend to explore soon is whether the information encoded and transmitted by single visual neurons changes as monkeys learn to differentiate visual patterns that they did not differentiate before.

Inferior temporal cortex and stimulus equivalence

According to our earlier unit-recording studies, nearly two-thirds of inferior temporal neurons receive information from both hemispheres (i.e., from both

the left and right visual hemifields) via the forebrain commissures. We have therefore proposed that inferior temporal cortex is important for stimulus equivalence across the visual fields. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we prepared monkeys with bilateral inferior temporal ablations combined with section of the optic chiasm and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either inferior temporal lesions alone or chiasm-section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkeys had to learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence across the visual hemifields depends on the convergence of their projections onto single inferior temporal neurons. We are currently testing a further prediction from this hypothesis, namely, that inferior temporal lesions will prevent transfer of a visual pattern discrimination from one locus to another within one hemifield. The method involves training monkeys whose maintained fixation is being monitored as described earlier to discriminate a pair of patterns in one quadrant of a visual hemifield and then test them for transfer to the other quadrant. Transfer should occur, as before, only if inferior temporal neurons are present to mediate the spatial convergence of visual information. In a related electrophysiological experiment (see preceding section) we have determined that, in fixating monkeys performing a discrimination parafoveally, single inferior temporal neurons are indeed activated by their adequate stimuli irrespective of stimulus position in their large receptive fields.

Not only inferior temporal but all areas within the cortical visual pathway are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of posterior temporal cortex, area TEO, like the more rostral temporal cortex, area TE, receive their contralateral input via the splenium and anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we have attempted to localize and to quantify their contribution to vision by application of the 2-deoxyglucose method. In this experiment, rates of local cerebral glucose utilization (LCGU) were measured throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

Results indicated that there were no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere. A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas, in animals with tract section only, LCGU

reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. To test whether the commissural input to the more posterior zone might be effective only against a background of activity provided by an intact retino-geniculo-cortical pathway, we prepared a series of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye, rather than by right optic tract section. Quantitative analysis, however, failed to reveal any greater level of activity in the new animals with a chiasm cut than in the earlier animals with a tract cut at either the OC-OB border or within areas OB, OA, or TEO of the visually deprived hemisphere.

These results have presented us with a paradox. On the one hand, our metabolic data on area TE are in good accord with existing anatomical data, which indicate that area TE receives interhemispheric projections through both the splenium and the anterior commissure. On the other hand, the same metabolic data give no indication of the equally heavy commissural projections that are known to be present in the more posterior visual areas.

A possible solution to this puzzle has come from our recent studies of neurons in area V4, the largest visual area in cytoarchitectonic area OA. As described in the preceding section, the receptive fields of V4 neurons have large, silent suppressive surrounds: Stimuli placed outside the receptive field are without effect themselves, yet they will often suppress the response to a receptive field stimulus (a proposed mechanism for figure/ground separation). Tests to determine whether these suppressive surrounds extend far across the vertical midline into the opposite visual half-field have been positive, and in preliminary experiments we have found that this suppression is nearly eliminated by section of the splenium. Thus, the commissural inputs to V4 appear to be largely suppressive, in contrast to the commissural inputs to TE, which are known to be at least in part excitatory. If neurons in the remainder of the prestriate complex also receive suppressive rather than excitatory inputs from the opposite hemisphere, this would help explain not only the metabolic data but also the well established finding that, despite heavy callosal interconnections between opposite prestriate areas, neurons in one prestriate area have excitatory receptive fields that are essentially limited to the contralateral half of visual space.

Inferior temporal cortex and object recognition

The spatial convergence upon single cells in area TE provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in fixation, distance, direction, and angle of regard that lead to stimulation of different populations of striate and prestriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the

effects of experience or training. We now have evidence that the inferior temporal cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Trained monkeys that are shown an object once will demonstrate that they recognize it as familiar several minutes later by avoiding it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent stimulus presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be area TE, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, area TE contains the traces laid down by previous viewing, and these serve as coded and stored representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. An experiment is now being designed to study these postulated visual traces at the single-unit level. The question to be addressed is whether or not a neuron that at first responds equally to two similar stimuli can be induced to respond differently to them by training the animal to attend to their physical differences.

A limbo-thalamic circuit and object recognition

In the process of investigating the role of other temporal-lobe structures in object recognition, we obtained a result that is particularly exciting because it may help to solve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies is that if damage to the hippocampus is combined with damage to the amygdala then a profound recognition loss ensues, a loss that is even greater than that produced by lesions of inferior temporal cortex.

Before the implications of this finding are considered, it should be noted that there was an alternative explanation for the severe effects of the combined lesion which needed investigation. That is, neither the amygdalectomy nor hippocampectomy had included all of the entorhinal cortex, whereas the combined lesion did. The results were therefore consistent with the possibility that the severe recognition loss after the combined lesion was actually due to destruction of the entorhinal cortex. A test of this possibility has now shown that even when total entorhinal ablation is combined with hippocampectomy the recognition impairment is not much greater than after hippocampectomy alone, whereas when total entorhinal ablation is combined with amygdalectomy the impairment is just about as severe as it is after combined

removal of the amygdala and hippocampus. In short, the entorhinal lesion appears to be equivalent to a hippocampectomy in this situation, presumably because it disconnects the hippocampus from the inferior temporal visual cortex. The results thus support our earlier conclusion that combined damage to the amygdaloid and hippocampal systems is necessary to produce a profound recognition loss in monkeys. That discovery has not only opened up a new possibility for resolving the discrepancy between clinical and animal findings but has also led to new insights into the neural mechanisms of memory.

According to our present model, object recognition depends on a cortico-limbo-thalamic pathway, activation of which leads to the storage in cortex of the trace or representation of the stimulus that gave rise to the activation. In the case of vision, as already indicated, the cortical tissue in which the storage is presumed to take place is area TE. However, as will be described in a later section of this report, other sensory modalities appear to be organized neurally along lines similar to those of the visual modality, and each is presumed to be represented by a highest-order processing station located in the anterior temporo-insular region with functions analogous to those postulated for area TE. We know that each of these highest-order sensory processing stations projects both to the amygdala and, via relays in the perirhinal and entorhinal cortex, to the hippocampus. Furthermore, we have shown that, in vision, one-trial object recognition is severely impaired not only by area TE lesions, or by combined amygdalo-hippocampal lesions, but also by disconnection of area TE from the amygdalo-hippocampal complex.

In our most recent studies, we have found that the limbo-thalamic portion of the pathway actually consists of two relatively independent limbo-thalamic segments, one from the amygdala through the amygdalofugal pathways (AFP) to the magnocellular portion of n. medialis dorsalis (MDmc), and the other from the hippocampus through the fornix (Fx) to the anterior thalamic nuclei (Ant. N). The evidence is based on comparison of the effects of separate and combined AFP and Fx transections, as well as of separate and combined MDmc and Ant. N ablations. In both cases, the combined lesions yielded significantly greater recognition losses than did the separate lesions. Because of their newly discovered functional significance, the two limbo-thalamic projection systems are now being mapped in detail with axonal transport techniques. The initial results show that the amygdaloid projections arise throughout the complex, though most heavily from the basomedial nucleus, sweep through the substantia innominata, and then travel in the inferior thalamic peduncle to enter the head of the thalamus before passing caudally to terminate in MDmc and n. reuniens; allocortical areas adjacent to the amygdala also contribute significantly to this projection. Hippocampal projections arise predominantly in the subiculum and terminate most heavily in nuclei anterior medialis, anterior ventralis, and lateralis dorsalis, with lighter projections to nuclei reuniens, centralis latocellularis, rotundis, and paraventricularis; all of these projections course through the medial part of the fornix, though n. lateralis dorsalis also receives a nonfornical input which runs through the medial pulvinar.

In view of the critical importance of the two limbo-thalamic pathways for recognition memory, we have attempted to learn whether their prefrontal targets might also be involved in this function. Our preliminary evidence indicates that combined damage to ventral prefrontal cortex (the target of MDmc) and adjacent anterior cingulate cortex (the target of Ant. N) does indeed produce a profound loss in visual recognition. The discovery of a possible functional relationship between the ventromedial prefrontal cortex and the limbic system provides an important new clue about the organization of prefrontal cortex. That is, for certain memory functions, the dorsolateral prefrontal cortex may depend on interaction with the ventromedial prefrontal region in much the same way that anterior temporo-insular cortex depends on interaction with the medial temporal region.

The cholinergic system and object recognition

In the recognition model that we have proposed, a stimulus leaves a lasting trace in the sensory modality's highest order processing station whenever that stimulus activates the cortico-limbo-thalamic pathway described above. This hypothetical circuit is incomplete, however, because it does not specify the link through which the limbic and thalamic structures reactivate the cortex that activated them. One possible candidate for this missing link is the basal forebrain cholinergic system. Several lines of evidence support this view. First, cholinergic agonists and antagonists have long been known to influence many forms of memory in many species, and in a recently completed study of our own we found that the same holds true for recognition memory in the monkey. In this study, monkeys given lists of 20 new objects to remember were able to recognize 75% of them reliably on list after list; the same monkeys then exhibited dose-related gains and losses, respectively, when they were pretreated with physostigmine and scopolamine. A second line of evidence comes from recent neuropathological studies in patients with Alzheimer's disease, who often show a marked memory loss as one of their earliest symptoms. In patients with this disease, a selective cell loss has been observed in both the nucleus basalis of Meynert (nbM) and the diagonal band of Broca (dbB), the parts of the basal forebrain that are the major source of cholinergic inputs to the cerebral cortex and limbic system. This selective cell loss was accompanied by markedly decreased cortical levels of choline acetyltransferase and acetylcholinesterase, enzymes involved in the synthesis and metabolism of acetylcholine. Third, the same effects, namely, decreased levels of these enzymes, has been produced experimentally by damaging the basal forebrain cholinergic system in animals. Finally, in some preliminary experiments performed in our laboratory in collaboration with investigators from the Johns Hopkins University we have found evidence of a recognition memory impairment following injections of the neurotoxin, ibotenic acid, into the basal forebrain system. When the damage was restricted to nbM, identified by electrophysiological recording, the impairment did not appear until the animals were challenged with the cholinergic antagonist, scopolamine, suggesting that the critical tissue had been damaged only in part and had thus simply rendered the animals more vulnerable to a pharmacological challenge. When the damage was extended in one animal to include both nbM and dbB,

however, the animal showed severe impairment in recognition memory without any drug challenge. Assuming that the effectiveness of such combined damage to the basal forebrain cholinergic system can be corroborated in additional animals, new studies will be undertaken to discover the anatomical routes through which limbic and thalamic structures might activate this critical system and thereby complete the postulated memory circuit.

Amygdala, hippocampus, and associative memory

Once the trace or representation of a stimulus has been stored in the highest-order processing station for any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory; according to our neural model, this reflects their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, but the amygdalectomized monkeys fell to chance performance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that although both the amygdala and hippocampus are important for associative memory, their roles are totally different. Many further analyses along the lines of these two experiments are needed, however, before the selective associative memory functions of the amygdala and hippocampus can be precisely identified. For example, the association of an object with an affective state, such as fear, pleasure, etc. appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view is being obtained in an experiment showing that one-trial object-reward association is impaired more by amygdaloid than by hippocampal lesions, although neither deficit approaches in

severity the one produced by combined removal of these two structures. By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

A cortico-limbic pathway in somesthesia

The work described above elucidating a cortico-limbic system for visual perception and memory has led to the search for analogous systems in other modalities. So far, analogous anatomical pathways have been found for both taste and audition, but because of early difficulties in establishing such a pathway in somesthesia, particular attention has been paid to this modality. Also, anatomical studies on the somatosensory system have been supplemented by neurobehavioral and, more recently, electrophysiological studies, in order to permit functional comparisons with the cortico-limbic system in vision.

Previous anatomical and electrophysiological studies had indicated that both the superior parietal lobule (area PE) and the second somatosensory area (SII) were possible relays in a processing pathway connecting the primary somatosensory area (SI) with the limbic system. To evaluate these possibilities behaviorally, monkeys with lesions of either area PE or SII were compared with normal controls in the ability to differentiate objects on the basis of texture, shape, and size. The results indicated that SII, but not area PE, is necessary for such tactual discrimination. This finding led, in turn, to the search for a pathway linking SII with the limbic system. Although our earlier experiments yielded ambiguous results, recent studies employing both anterograde and retrograde axonal transport techniques have provided positive evidence for such a pathway.

Injections of cell markers were made into the hand representation of physiologically identified cortical fields lying in or near the lateral sulcus of the monkey. These regions include SII, area PF, the retroinsular cortex (Ri), and the granular and dysgranular insular fields (Ig and Id). The results revealed reciprocal connections between SII and Ri, SII and area PF, SII and Ig and Id, and Ri and Ig. In addition we have confirmed the previously reported reciprocal connections of SI and SII, and demonstrated reciprocal projections between area PE and both Ri and area PF.

The laminar pattern of termination of these projections suggests the sequential order in which the somatosensory fields process information. In the visual system, forward corticocortical projections (i.e. outward from striate cortex) are characterized by a heavy input to layer IV, while backward projections (i.e. towards the striate cortex) completely avoid layer IV and instead have a characteristically heavy input to layer I. By analogy with the visual system, an analysis of the laminar pattern of termination in our material indicates that the forward sequence of projections is: SI to SII and area PE, area PE to Ri and PF, PF and Ri to SII, and SII and Ri to Ig; SII also projects to Id. Finally, we have confirmed previously reported

projections from Ig and Id directly to the amygdala and indirectly to the hippocampus via the rhinal cortex. These data demonstrate that a pathway is available for relay of somatosensory information from SI through SII to the insula, and from there to the limbic structures of the temporal lobe

In the course of working out this ventrally directed cortico-limbic pathway for touch, we simultaneously resolved a long-standing difficulty for the functional aspect of the hypothesis which postulates that SI and SII process tactile information in sequence. The difficulty for this hypothesis was the earlier supposition that both SI and SII were projection zones of the primary somatosensory relay nucleus, n. ventralis posterolateralis, implying that the two cortical areas received and processed tactile information in parallel. Our new evidence has corrected that supposition by revealing that SII receives its major thalamic afferents not from the primary relay nucleus as SI does but from a different cell group, n. ventralis posteroinferior. This is a pattern of projections that is consistent with a sequential corticocortical pathway. To test the proposal directly, however, we are currently examining the effects of ablation of SI on the responsivity of neurons in SII. If the notion is correct, SII neurons should no longer be activated by tactile stimulation.

Since the insula now appears to be an important link in the cortico-limbic pathway for touch, and since virtually nothing is known about the response properties of insular neurons that receive somatosensory afferents, we have begun to examine these neuronal properties in awake monkeys. So far, over 200 neurons have been examined in the posterior two-thirds of the insula, which is the portion comprising the granular and dysgranular insular fields. About one-third of the neurons could not be activated by any of the types of stimulation that were tried, but of the remainder nearly all responded to somatosensory input, with only two or three cells responding to auditory or gustatory stimulation, and none to visual. The insular neurons resembled those in SI and SII by responding both to cutaneous (75%) and deep (25%) stimulation, but not to their convergence, and by responding with low thresholds to stimulation of discrete fields. The insular neurons differed from the other cortical somatosensory neurons, however, in having larger receptive fields, with the great majority of them being bilateral. The latter properties resemble those of area TE neurons in vision and thus give further support to the proposal that the cortico-limbic pathways in the two modalities are organized in a parallel fashion. Nevertheless, one of the most critical tests of this proposal remains to be performed, namely, the test of whether removal of the insula will produce an impairment in tactile memory similar to that produced in visual memory by area TE lesions. Hints that the outcome will be positive were provided by earlier demonstrations that combined removal of the amygdala and hippocampus produce equivalent memory impairments in both vision and touch, and there seems to be no plausible alternative at present to the supposition that these limbic structures depend on the insula for their tactile input just as they were found to depend on area TE for their visual input.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. Soon after discovering this phenomenon in tests with objects we found that the same dissociation between impaired recognition but spared discrimination learning despite 24-hour intertrial intervals held for pictorial stimuli as well. This paradoxical success in the presence of severe memory loss implies the existence of an important retention mechanism outside the limbic structures of the temporal lobe.

We have since performed additional experiments to characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research. For example, preliminary evidence regarding the effects of damage to inferior temporal cortex (area TE) indicates that this cortical region is important not only for the limbic memory system but also for the nonlimbic habit system. Our aim now is to identify the subcortical structures belonging to the habit system, with our initial target being the corpus striatum.

Ontogenetic development of memory and habit formation

On the evidence that in the adult monkey there may be two relatively independent systems for retention of information, we recently initiated a series of studies to assess the development of these two systems in infant monkeys. Results thus far indicate that one-trial recognition, requiring memory of one object at a time for only 10 sec each, is absent in infants

younger than four months of age and does not reach adult levels of proficiency even at one year. This slow ontogenetic development of recognition memory was shown even more strikingly with longer delays and lists. In sharp contrast, when 3-month-old infant monkeys were trained on object discrimination habits, they performed exactly like adult monkeys in both acquisition and retention, even though intertrial intervals lasted 24 hours. These results strongly suggest that the two systems of retention that were found to be relatively independent in the adult monkey are also developmentally dissociable. Indeed, they provide evidence that the mysterious phenomenon of infantile amnesia could be due to the absence of a functional memory system in early childhood.

On the basis of this evidence, we have begun to prepare monkeys with neonatal removal of the limbic system (i.e. combined amygdalo-hippocampal removals) in an attempt to see how cognitive, emotional, and social behavior develops in animals whose amnesia might persist from infancy through adulthood. Animals with neonatal removals of area TE are serving as controls. The results so far indicate that, at two months of age, monkeys with neonatal limbic lesions display abnormal social behavior, whereas the operated controls are essentially the same as normal infants. Similarly, at three months of age, the infants with limbic lesions are impaired in habit formation (even though the same lesion in adulthood leaves the habit system intact), whereas the operated controls showed significant functional sparing of the system (compared to the effects of area TE lesions in adults). Our tentative conclusion is not only that early and late brain damage have different consequences but also that the direction of the difference depends on whether the damage is cortical or subcortical. That is, early cortical damage may be less disruptive than late, while early subcortical damage may be more disruptive.

Our plan is to follow the psychological development of our neonatally operated animals into adulthood. The results of the study will help to evaluate two provocative proposals from the clinical literature, namely, (a) that early dysfunction of the limbo-thalamic memory system could be one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology, and (b) that the reason a pure case of amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult.

In tandem with these developmental studies of behavior, we have begun to trace the functional development of the visual system metabolically, by applying the 2-deoxyglucose method to infant monkeys in a manner similar to that described earlier for adults. Thus far, a series of animals with unilateral optic-tract section combined with forebrain commissurotomy have been studied at various ages ranging from two days to six months. The results show that there are systematic age-related changes in both the absolute levels of LCGU within the seeing hemisphere and the relative levels of LCGU within the visually deprived hemisphere. Specifically, in all cortical visual areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and

then declined in the 6-month-old subject to levels found in adults. As in adults, the intact hemisphere of infants showed a progressive decline in LCGU along the ventral cortical visual pathway from a high in area OC to a low in anterior TE. This gradient was present in all infant subjects, but was shallowest in the two youngest.

In addition, the deprived hemisphere showed reduced LCGU relative to the normal hemisphere in all areas of the cortical visual pathway at all ages. Also at all ages, hemispheric differences were greatest in area OC and smallest in anterior TE. These differences, however, varied systematically with the age of the animal. Thus, for each cortical area, the relative difference between the normal and deprived hemispheres was smallest in the youngest subjects and approached the differences seen in adults only at about four months of age, the time at which LCGU appeared to peak. This finding that adult levels of metabolic activity are not reached until about 4 months of age is consistent with the behavioral data just described indicating that the neural capacity for visual object recognition is probably not developed until about this time.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve memory and other cognitive processes. (1) Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierarchically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal limbic system. Before reaching the motor system, the dorsal pathways from the several modalities converge in polysensory areas, which are critical for sensory attention, spatial perception, and motor guidance. The ventral pathways, by contrast, remain modality specific throughout their course and are important instead for stimulus recognition and stimulus meaning, and ultimately for triggering the motor response. (2) Stimulus recognition depends not only on stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects directly to the amygdala and indirectly to the hippocampus, and these two limbic structures project in turn to the medial thalamus. Storage of the central representation of a stimulus occurs only if this cortico-limbo-thalamic pathway is activated. Damage to this pathway results in recognition failure, a core symptom of amnesia. (3) Once the central representation of a stimulus has been stored, it can enter into association with the stored central representations of other stimuli and other events, thereby providing the stimulus with meaning. Thus, stimulus-stimulus associations appear to depend on cortico-amygdalo-cortical interactions, while stimulus-affect associations probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations seem to depend on cortico-hippocampal interaction; in this case

the hippocampus may act as the site of converging information from both the ventral 'stimulus recognition' pathway and the dorsal 'spatial perception' pathway, the latter involving projections from the polysensory areas through the cingulate gyrus to the hippocampus. Finally, stimulus-act associations could depend on interaction, via the limbic system, between the ventral and dorsal pathways within the lateral prefrontal cortex, which interacts in turn with the motor system. (4) Destruction of the limbic portion of the memory system does not affect all forms of learning and retention, however. At least one form, which has been labeled habit formation, remains nearly intact, presumably reflecting the operation of a powerful nonlimbic mechanism for retention of stimulus-response connections. On the basis of behavioral developmental studies, this nonlimbic habit system appears to mature considerably earlier than the limbic memory system.

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Annual Report of the Laboratory of Developmental Psychology

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Marian Radke-Yarrow, Chief

Summary

"Understanding of the dynamic order of living systems must be approached by the unprejudiced and unrestricted exploration of all aspects which a living system offers, evaluated logically and soberly... No single branch of life sciences holds the master key to the understanding of the common core of principles of life from which it has branched off; claims to the contrary stem from limited perspective, no matter whether they come from the molecular or evolutionary or the psychological or ecological or any other branch."

Paul Weiss ---- Developmental Biologist

The need for investigation at multiple levels of functioning, as expressed in Weiss's words, is nowhere better illustrated than in the search for understanding of developmental processes--the evolution of functions and properties from infancy to maturity. Fully adequate explanations of child development require linkages of knowledge across many levels of analysis (genetic, neural, behavioral). The focus of this Laboratory is on the behavioral level. The problems that are chosen for study fulfill several criteria. The first is their relevance to mental health problems and pathologies in development. The second involves our current state of knowledge: In what particular problem areas in biobehavioral research is investigation moving forward with considerable excitement, in which the elucidation of behavioral processes makes a significant contribution, and in which linkages of knowledge are possible and necessary?

The research endeavors of the Laboratory are devoted to problems of children whose behavioral integrity is at risk. These are (a) children whose parents suffer serious mental illnesses, (b) children who show early signs of problem aggression and an array of other behavior problems, (c) children negotiating adolescence, and (d) children confronted with serious medical problems. The etiologies of pathology as well as of successful mastery of potentially pathogenic conditions are research foci. Normal samples are always integral parts of the studies, allowing us, thereby, to address basic questions of normal child development.

Our research on children includes studies of behavior expressed in cognitions, affects, interpersonal interactions and relationships--elements by which healthy functioning as well as disorders and deficiencies in human functioning are manifested and by which treatments are evaluated. Development, per se--the courses, plasticities, stabilities, and discontinuities--is of central interest. A major concern is with the environments that impact on the child--how the child's behavior is linked to the world as well as to its chemistry. Another activity is exploration of collaborations with developmentally oriented researchers within and outside the Institute, to formulate a set of guiding ideas that will be the basis of research that begins to link neurobiological indices and measures of behavioral organization in the child.

The major ongoing research projects of the Laboratory are summarized below:

Rearing and Pathology

Depression is one of our most prevalent mental health problems. Such affective disturbance influences the lives not only of the millions of adults who have the disorder but also the millions of offsprings of affectively disturbed parents. Very little research has been carried out on children of depressed parents.

A program of coordinated studies is designed to investigate the transmission of adaptative and maladaptive behaviors in children in families with and without parental depression. We know that a certain percentage of the offspring of depressed parents develop depression. Is there anything about the behavioral repertoire of parents and children that increases the chances of depression in the offspring? Are there rearing conditions that inoculate the offspring? A fundamental part of this research has been the development of sensitive procedures for assessing the rearing environment and the psychological status and behavior of the child. A core paradigm has been developed by Drs. Yarrow, Kuczynski and Waxler which utilizes naturalistic and experimental methods. Mothers and children are observed with each other over a series of days. Observations are made when in a research apartment where conditions allow and encourage usual routines, behaviors, and feelings. Much of the structure of the time is of the participants' own making (playing, disciplining, eating, separating, getting upset, enjoying something). An underlying experimental structure imposes standard events which are likely to elicit certain classes of responses. Mother is observed with her 2-year-old and 5- to 8-year-old child. In addition to observations, data on belief systems, reports on support systems and life experiences, and psychiatric assessments are obtained. The same procedures, modified appropriately to take developmental changes into account, are repeated after an 18-month interval. Fathers are included in the follow-up observational assessments.

Families in this study include mothers diagnosed as normal or as having bipolar, major unipolar, or minor depression. Before primary analyses can be performed, substantial data collection must be accomplished. At this time, 80 families have been seen through the entire first battery of procedures. Follow-up is beginning. Preliminary findings from partial data and ancillary studies begin to yield information on the functional deficits of affectively ill mothers when they are outside acute illness states. Differences are found between bipolar and normal mothers in their orientations toward their children and in their rearing objectives, particularly on dimensions concerning affect. The affectively ill mothers compared with normals are more likely to have negative feelings about their children, but less likely to be open in their expression of feelings. They are more likely to be highly protective of their children; less likely to encourage openness to new experiences. The ill parents more often than the normals stress the child's control of feelings. Group differences are only a first level of analysis. Affective patterns are now being tracked in more detailed analyses,--such as interactions of affection between mother and child, and profiles of emotion expressed by mother and child (Drs. Waxler, Yarrow, Kuczynski, Friedman).

Analyses are underway on rearing functions that concern the management or control of child behavior and the teaching of the child. There are limited scientific data on these functions, particularly with regard to how they are disordered by parental pathology. Characteristics of parental control (the content, purpose, and affective quality of parental control behaviors and their effects on the child) are being analyzed (Drs. Kuczynski and Yarrow). The cognitive content of the rearing environment (teaching and verbal communications between parent

and young child, inferential abilities of mother and child, attributions made by mother) are being investigated in normal and depressed mothers (Drs. Friedman, Iannotti, Pierrehumbert, Cummings, Waxler, Wylie).

Analyses of the children's functioning in relation to parental pathology are still too incomplete to allow more than tentative findings. In our earlier work with manic-depressive parents, their children were found to have multiple disturbances in interpersonal relationships and in emotional regulation. In analyses now under way, unipolar major depressive and minor depressive mothers as well as bipolar mothers are compared with normal mothers. To date, there are indications that many of social-emotional problems of children of manic-depressives do not typify children of unipolar and minor depressive mothers, with one exception. Children (2-year-olds) with a depressed mother, regardless of type of depression, are consistently less likely than children of normal mothers to show physical aggression in relating to peers (Drs. Waxler, Cummings, and Hay). Sibling relationships also are being explored. If a mother is depressed, some of the responsibility for caregiving and mediation of conflicts falls to the older sibling. This has implications for children's psycho-social development (Drs. Waxler, Hay, Yarrow, and Friedman).

Sensitive diagnostic instruments for use with children from 1-1/2 to 5 years of age have not been available. They are essential to the exploration of early precursors of later disturbances in behavior. An instrument for these ages has been developed and is being tested (Drs. Cytryn, McKnew and Hay). This assessment should contribute to a developmental view of affective illness and to attempts in secondary prevention.

It will be possible to compare different procedures for assessing children. The child's diagnosis (as obtained by child psychiatrists) can be examined in relation to (a) the mother's assessment of the mental status of the child (Achenbach) and (b) behavioral observations of the child made over several days of time in the laboratory apartment. This composite of information will provide a considerably more developed profile of the differential competencies and vulnerabilities of children with and without an affectively disturbed mother than is usually obtained clinically or in research (Drs. Yarrow, Cytryn, McKnew, Waxler, and Kuczynski).

Another type of parental dysfunction, namely parents who physically abuse their children, is investigated by Drs. Trickett and Susman. The objectives are to examine the development of abused children, the childrearing environment of abusive families with particular attention to processes that lead to abuse, and the relations between given childrearing variables and the child's patterns of behavior. Again both naturalistic and experimental approaches are used. The child, mother, and father are seen in the Laboratory. Parents receive psychiatric interviews.

Preliminary analyses document an expected increased incidence of behavior problems among children of abusing parents. This is the case especially for externalizing types of problems, e.g., aggressive and/or antisocial behavior, but it is true also for internalizing problems, e.g., depression, withdrawal, somatic complaints. In a social problem-solving test, abused children are more likely to suggest forceful solutions to interpersonal problems than are the control children. Data on childrearing attitudes and disciplinary practices show that abusive parents believe in, as well as use, harsher punishment than control families, although the great majority of both groups use physical punishment at times. Other

preliminary analyses show more abusive mothers as being currently depressed and having smaller social networks than control mothers. The relationship does not hold for fathers. This study addresses two distinct etiological issues. One focus is on the causes of child abuse with particular emphasis on the role played by parental psychopathology and parental child-rearing attitudes and behavior. The second focus is on the effect of child abuse on the psychological development of the victims. Information on these two issues can aid greatly in the development of treatment programs for abusive families and preventive interventions.

Origins of Aggression

In the research of Drs. Cummings, Waxler and Iannotti, normal and atypical patterns of aggression and empathy, and the modulation of affects in young children are investigated in a longitudinal study. Fifty 2-year-olds were observed with peers and with adults in repeated sessions of play in the laboratory. The procedures are now being repeated when the children are 5 years old. Patterns of aggression appear to be quite stable for many of the boys; not for the girls. Good measures of aggression require more than frequency counts; the patterns of intensity, escalation, lability of expression and frequency provide more diagnostic indices. Profiles of the child's behavior in adult and peer systems of interaction are examined with attention to developmental characteristics, the environments in which the child is reared, and, extending Dr. Waxler's earlier work, mother's psychiatric status.

Mothers' retrospective reports of their children's irritability, anger, and aggression in the home at 18 months were significantly related to the children's aggression with peers, measured in the laboratory at 26 months. Mothers who reported high use of admonishment, scolding, and physical restraint as discipline at 18 months had children who show high aggression to peers at 26 months. These data suggest a very early cycle of parent-child aggressive interchanges (Drs. Trickett, Waxler, and Yarrow).

Early Adolescence

Two projects in the laboratory focus on psychological functioning in early adolescence. Adolescence is recognized as a period of rapid and fundamental biological and psychological change, and also a time in which serious psychological problems appear with increased frequency. The first study (Drs. Susman, Nottelmann and Blue) examines closely the role of endocrine and physical growth changes in relation to cognitive, emotional, and interpersonal behavior. Parental rearing influences are examined in relation to both classes of variables. Boys and girls (9 to 14 years) are followed over a one year period. An extensive battery of psychological and biological measures is used, including adolescents' self-assessments, parents' assessments of their child, observations of parents and adolescent interacting together, and biological laboratory assessments of Tanner growth stage and blood samples for levels of gonadotropins, gonadal steroids, and adrenal androgens. Analyses of data have just begun. Aggressive behaviors and their relation to biological and environmental factors are one specific interest. There is evidence of a sex difference in the stage at which aggression and uncontrolled behavior become problems; for girls it is at an earlier stage of puberty than for boys. Aggression in relation to testosterone levels is being analyzed.

In a second study of early adolescence, Dr. Nottelmann has examined the psychological functioning of 11- to 13-year-olds as they encounter both the physiological changes of puberty and the environmental transition from elementary school to middle or junior high school. The children are studied at the end of elementary

school and followed up at a later point after transition. Children's self-reports, peers' and teachers' appraisals and parents' reports are the primary data sources. Early analyses provide a provocative critically disruptive profile of life events superimposed on the normal transitions in this period of childhood. These children from a generally stable and privileged suburban population have experienced many major disruptions beyond their control: Almost half reported illnesses of their own or of family members; almost half experienced significant separations from friends or family. In 31% of the cases, a death had occurred in the family. Potential or present psychological problems in a not insignificant minority of young adolescents are flagged by feelings of low self-esteem and marginal peer relationships (15 to 20%), by involvements in fighting and bullying in school (18%), and by having been punished in school (27%). Thus in a "privileged" part of the population, young adolescents must cope with an accumulation of problems from many sources. Analyses will profile the lives of adolescents who have low self-esteem, poor peer relationships, and problems of aggression in school.

Biological Conditions and Psychological Status

Some of the research in the Laboratory is concerned with direct linkages between specific biological (deficit) conditions and psychological status of the child. One study has followed up the effects on the child of having had, as a primary source of nourishment for a period in infancy, a commercial infant formula found to be chloride-deficient. The children had developed metabolic alkalosis. Psychological assessments made at 24 months of age showed some lowered cognitive and motor-perceptual abilities (compared with a sibling control group). There were, however, no remaining deficits at a 42-month follow-up (Dr. Moss).

A second study continues earlier work in the Laboratory in which it was found that CNS treatment of child patients with acute lymphocytic leukemia had a delayed effect of producing a deficit in intellectual functioning that appears to be somewhat progressive. On the basis of this research, a program was undertaken to compare the long term effects of the CNS treatment with an alternative non-CNS treatment. Patients are tested on a series of neurophysiological measures (memory, attention, perceptual-motor functioning, coordination, concept formation, and general cognitive skills). They are tested twice over a 5-year period. This study is done with the collaboration of National Cancer Institute and various children's hospitals throughout the country (Dr. Moss).

A third study looks at the development of premature infants. When an infant is born prematurely, its liver does not produce adequate quantities of the enzyme that detoxifies the bilirubin that is present in the blood stream. Consequently, the bilirubin crosses the blood brain barrier and affects various brain areas.

High levels of bilirubin may produce severe cognitive, affective, sensory, and motor deficits. It is reasonable to assume that less extreme levels may have more subtle effects in these same areas. Therefore, 45 low medical risk pretermatures were studied a month after treatment for jaundice. Neurological functioning was evaluated using the Parmelee Neurological Exam and recorded observations of state, state-change, and activity. Physiological jaundice was found to be predictive of a depressed visual responsiveness, greater sleepiness, and reduced state-change many days after jaundice cleared. Findings lead to questions about the specific mechanisms through which bilirubin affects later visual responsiveness and state change in pretermatures; also about the timing of phototherapy (preventive or responsive phototherapy) in the treatment of young preterms (Drs. Friedman, Werthman, M. Waxler, C. Waxler).

Dr. Kosawa, who is visiting from Japan, is carrying out a cross-cultural study of mother and infant behavior. In earlier work (Caudill and Weinstein) cultural differences were reported. American mothers emphasized distal behavior toward their infants and the infants tended to be active and vocal, whereas Japanese mothers tended to have more close physical contact with their infants who tended to be quiet. Dr. Kosawa is replicating this study and also assessing infant temperament as a factor in the cultural differences.

Staff and Staff Associates

This year has seen some stabilization of staffing after a disruptive year involving changes brought about by reduction-in-force proceedings. We have had two foreign scientists visiting in the Laboratory, Dr. Blaise Pierrehumbert from the Swiss National Foundation for Scientific Research, Bern, Switzerland and Dr. Yorio Kosawa from the Ministry of Education, Kobe-city, Japan. We have been most fortunate in having them here. Both have carried out projects relating to our ongoing studies. We have had, also, the benefits of the special expertise of Drs. Dale Hay (State University of New York, Stony Brook) and Ruth Wylie (Goucher College, Towson, Maryland) who have been at the Laboratory for limited periods as Guest Workers. As in years past, the Laboratory has attracted graduate and undergraduate students from nearby and distant colleges and universities. These students have participated in significant ways in many of the research projects.

Dr. Jerome Blue who has completed a Staff Fellow appointment has been selected as a Congressional Fellow for the coming year.

Scientists in the Laboratory hold a variety of committee positions in both national and international scientific societies, and are on editorial boards of major journals of the discipline. They have been well represented on the programs of the national and international meetings. In addition, they serve on committees within NIMH, ADAMHA, and NIH.

The Future

As already described, many of the projects involve extensive data collection which must be accomplished before major findings can be reported. Most of the projects are now sufficiently mature in data for meaningful analytic work. We would expect to have many fruits of these analyses in the coming months. As recipients of a MacArthur Research Network Award, a number of investigators in the Laboratory have participated in Network research conferences at the University of California, San Diego. Plans are underway for collaborative research with scientists in the Networks at Harvard and the University of Colorado. These will be studies of emotional development in young children.

Annual Report of the
Laboratory of Psychology and Psychopathology
National Institute of Mental Health

October 1, 1982 to September 30, 1983

Allan F. Mirsky, Ph.D., Chief

This is the third full year report of the program of the Laboratory of Psychology and Psychopathology. In addition to the progress in research, which is reported below, there have been two major additions to the Laboratory of Psychology and Psychopathology this year: the Clinical Infant Research Unit (CIRU), and the Section on Clinical Brain Imaging (SCBI). These are headed, respectively, by Dr. Stanley I. Greenspan and Dr. Robert M. Cohen. The CIRU was formerly contained within the Mental Health Center that was given a new mission during the RIF in DHHS which took place in 1981. The hope is that the work of the CIRU, both administratively and substantively, can be encompassed and integrated within the ongoing programs of the Laboratory of Psychology and Psychopathology. The CBI Section was formerly part of the Biological Psychiatry Branch (Branch Chief Dr. William Bunney) under the leadership of Dr. Monte Buchsbaum. With the departure of Drs. Bunney and Buchsbaum, the CBI group, which has the responsibility for conducting PET studies within the NIMH was transferred to the Laboratory of Psychology and Psychopathology.

A transitional report from the CIRU is appended to this Summary; the work of the CBI Section is summarized as a project report. We have at last occupied our permanent laboratory space in the ACRF in May 1983; since acquisition of some of our necessary research equipment is still incomplete and since renovation of our laboratory has still not been accomplished, the present summary report is in large part a recapitulation of last year's report. However, we have made extensive contacts with organizations and persons both within and without the NIMH with respect to access to patient groups (schizophrenic, epileptic and brain-injured subjects), and we stand poised and ready to implement the human studies referred to below. The scope of the animal model studies described in the subsequent section is more restricted than had been planned; however, a number of studies of attention in the monkey are in progress.

A brief outline of studies appears below.

A. Human clinical studies of attention disorders

1. The taxonomy of attentional disorders
2. Brainstem mechanisms in attention impairment
3. Neurobehavioral studies in absence epilepsy

B. Animal models of attention disorders

1. Experimental models of generalized seizures of the absence type
2. Analysis of cellular activity in the attention system within the monkey brain
3. Brain lesion and state change effects on visual attention

C. Psychobiology of cognition

1. Studies of depression
2. Studies of dementia
3. Learning disabilities
4. Pharmacological studies of cognition

D. Autonomic nervous system activity in attention and psychopathology

E. Individual differences in the study of heredity and environment in schizophrenia

F. Cognitive, perceptual and nosological studies in depression

G. Clinical Infant Research Unit -- Transition Report

Introduction

The basic new thrust has to do with the creation of a program of research aimed at developing a taxonomy of attentional disorders. We aim to describe in detail, at a number of levels of scientific analysis, the nature of the system within the brain that provides the substrate for attention, the basic organism/environment interaction. The system involves the participation of many elements within the neuraxis, from phylogenetically ancient structures within the mesencephalon and pons through structures in the diencephalon including some regions of hippocampus, through regions of the temporal, parietal, and frontal cortex. The program of research necessary to describe the attentional system and its manifold dysfunctions involves the study of a number of model or paradigmatic clinical conditions. These include patients with prefrontal, temporal, or inferior parietal lesions. Such cases represent perhaps the clearest instances of brain-behavior relation in that they involve the behavioral effects of specific lesions which can be documented anatomically with a reasonable degree of accuracy. In some of these cases, i.e., lesions including the right inferior parietal lobe, the attentional loss is most pronounced and obvious: the patient may neglect the entire left side of space contralateral to his lesion. The mechanisms underlying the loss, however, are not particularly well understood.

The next most difficult level of analysis involves study of patients with epileptic disorders of the generalized or absence variety. During the absence attack, which some of these patients display in relatively pure form (uncomplicated by even minor motor seizure manifestations), there can be total abeyance of consciousness for varying periods of time. Such absence periods are usually accompanied by dramatic changes in the EEG: generalized, bilaterally synchronous slow discharges of the three per second spike and wave (SW) variety. These discharges allow a specific point of entry into the study of the impaired attention or consciousness; experiments can be designed to analyze behavior or stimulus processing during, preceding, and following SW discharges, so as to facilitate understanding of the nature of the pathological brain processes involved in the absence attack. The electrographic sign (the SW discharge) thus provides in some sense a temporary lesion which can be the subject of neuropsychological study. Although it is less satisfactory as an experimental variable than

the existence of a documented loss of tissue, absence epilepsy does at least provide the opportunity to study each patient as his own control. The brain-injured and epileptic cases provide models and paradigms for the experimental analysis of attention loss which may help in the understanding of the symptoms of impaired attention in clinical states in which the neuropsychological nature of the disturbance is more obscure or not well understood. Instances of these include schizophrenia, infantile autism, and the various types of dementia (e.g., Alzheimer's disease, Korsakoff's syndrome, Huntington's disease). For many of the clinical states under study, the nature of the human clinical material ordinarily precludes the use of certain kinds of invasive experimental methods (e.g., deep probes or implanted electrodes, brain biopsies). Questions arise often as to the locus or nature of the lesion process in absence epilepsy, for example, which can best be approached, given current levels of technology, with animal models. Thus, the role of lesions or other perturbations in specific subcortical brain regions in absence epilepsy can be explored in animal models. Monkeys can be trained to perform complex attention-demanding tasks, and experimental modifications of the animal's brain can be performed: lesions, either static or irritative, of various subcortical structures; electrical or direct chemical stimulation; administration of neurotransmitters, hormones, enzymes, or blocking agents. The effects of such experimental agents in trained, behaving animals can lead to more refined hypotheses about the nature of pathophysiological processes; moreover, models for testing the efficacy of new therapies may be provided by this work. Aside from models of pathophysiology, however, the highly advanced and complex brain of the monkey affords an opportunity for study of the basic phenomena of attentional brain function in primates. A considerable program of work can thus be followed profitably, leading to analysis of neural processes, both at the level of the individual nerve cell and the nerve network, involved in attention. The broad outlines of projects which will be pursued by the LPP are described below in section B.

A. Human clinical studies of attention disorders

1. The taxonomy of attention disorders: A collaborative project

Many of the members of the LPP are involved in a joint project in which a wide variety of attentional, cognitive, autonomic, and electroencephalographically-derived tests are applied to the same populations of experimental and control subjects. These will include epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them and will lead to a better neuropsychological characterization of their impairment.

There are four major elements within this program that will be discussed at this time: the work of the Cognitive Psychophysiology Unit (Dr. Connie C. Duncan-Johnson, head); the Imaging Studies of Dr. Richard Coppola; the Generalized Attention Test (GAT) development of Drs. Nakamura and Coppola; the development and implementation of the Neuropsychological Test Battery by Drs. Goldberg (consultant), Dr. Duncan-Johnson and Ms. Squillace. Dr. Duncan-Johnson's Unit will apply techniques of cognitive psychophysiology to elicit and evaluate the several event related potential components (auditory and visual) which have been

shown to relate to attention, uncertainty and surprise (P300, N140, etc.). Similar experimental paradigms (e.g., the "odd-ball" method) will be applied to various groups of subjects with attentional disorders so as to be able to compare and contrast ERP component amplitudes, latencies and distributions. It is anticipated that these studies will help to illuminate (in conjunction with other methods) the nature of the similarities and differences among various attentional disorders.

Dr. Coppola's imaging laboratory became operational in the fall of 1982. Continuing from the work begun with Dr. Buchsbaum a 32 lead continuous EEG mapping system was implemented. The laboratory is now regularly recording 32 lead studies from a variety of patient groups. It appears that in some situations the additional imaging resolution gained from 32 leads vs 16 on one hemisphere can be quite useful. In other cases the 32 lead capability is used to record from both hemispheres simultaneously, allowing bilateral comparisons. Aside from evaluation of this new technique, studies are in progress examining patients with depression (under IV procaine HCL challenge); examining cases with presumed Alzheimer's disease; and developing EEG imaging in patients with epilepsy.

One of the behavioral measures to be used in this study is the Generalized Attention Test (GAT) which is being developed at the NIMH by LPP staff. It represents an extension and modification of the Continuous Performance Test (CPT) of attention which was originally refined and perfected at the NIMH during the period 1954-1961. The CPT has been used extensively by various groups of investigators, particularly in the study of epilepsy, schizophrenia, and metabolic illnesses. The GAT extends the concept of the CPT to involve a variety of cognitive dimensions (such as intra and inter-dimensional shifts), as well as parametric control of other perceptual variables. The GAT is expected to prove useful in exploring the dimensions of attention impairment in the several clinical groups under investigation in this taxonomy study. The GAT is designed for use with both humans and monkeys so that direct comparisons can be made of the results in each species. Preliminary results on humans indicate that evoked responses during performance on the GAT differentiate well between attention conditions. Also, monkeys have been successfully trained to perform on this task.

The Neuropsychological Test Battery was developed by various LPP staff, including Dr. Connie Duncan-Johnson, Ms. Kathleen Squillace and outside consultant Dr. Elkhonon Goldberg of Einstein Medical School. After considerable discussion, review of the literature and consultation with IRP staff, a battery of neuropsychological tests was devised which provides a reasonably complete assessment of the various executive, mnemonic, linguistic and attentive capacities of the human brain. The test battery has been administered to about 12 cases so far, and the information it provides will form the neurobehavioral data base which will be used to interrelate, evaluate and integrate the various electrographic, biochemical and other physiological measures applied to attention disorder patients.

2. Brainstem mechanisms in attention impairment

There are a number of lines of evidence leading to the inference that the classical brain stem systems implicated in attention (in the vicinity of the mesencephalic and pontine reticular formation) are damaged or malfunctioning in

certain clinical syndromes. These include absence epilepsy, infantile autism, and possibly, some forms of schizophrenia. This project aims to use existing measures of auditory and somatosensory brainstem functioning which were developed for assessment of neurological (and especially demyelinating) disorders to assess the integrity of these structures in the several clinical conditions.

3. Neurobehavioral studies in absence epilepsy

In this project, specific sensory tests are used to assess functioning before, during, and following absence attacks in patients suffering from generalized seizures of the absence type. This work is done in conjunction with computer-assisted analysis of EEG changes prior to the appearance of SW discharge and has led to an appreciation of the changes in the energy (or more precisely the power) spectrum in cortical areas immediately preceding a paroxysmal EEG discharge. The findings already obtained in this project, which was begun at Boston University, may be considered as elements that can be incorporated in the development of a prosthetic device in treatment of some forms of epilepsy. That is, the EEG changes could be monitored and used to activate a device which would forewarn a patient of impending absence attacks. With help, patients might be able to develop strategies which could prevent, arrest, or abort such attacks. While it is true that most persons with absence seizures can be helped with relatively mild forms of anti-convulsant medications, it is not true of approximately 20% of such cases. These persons require either multiple anti-convulsant drug regimens or near-toxic doses of such drugs. Moreover, even low doses of anti-convulsant medications (whether considered to be easy to tolerate or not) may have deleterious side effects on behavior and biological capacities, especially when they are ingested by young, developing persons for periods of years. Excellent patient sources have been identified, and the cooperation of referring units has been secured. All of the hardware for this project has been secured and substantial progress is expected during the next year.

An additional component of this project to be pursued this coming year involves neurochemical studies. Neurochemical investigations of major psychiatric disease such as depression and schizophrenia have been disappointing. This may be for the reason that the causes of these diseases are heterogenous and not reflected in just one change in one neurochemical system. It may also be the case that the influence of thought and mood on neurochemical variables is rather subtle compared to the influences of other systems. The investigation of more homogeneous disorders which involve systems that are relatively better understood may offer more immediate rewards. Such studies may also help us to understand more traditional psychiatric disorders. Anorexia nervosa is one of these homogenous disorders; another may be absence epilepsy. Pharmacological studies in absence epilepsy have found that medications that are clinically effective in preventing seizures act either on GABA or catecholamines. Absence epilepsy may involve a disturbance in the regulation and interplay between catecholamine arousal mechanisms and GABA inhibitory effects. This may be reflected in the "cortico-reticular" disturbance (as posited by Gloor) that underlies absence epilepsy. We intend to test this hypothesis in a number of ways. We will measure CSF during states of few absence seizures and during states of many seizures to determine if there are alterations in concentrations of catecholamines, GABA, and other related neurotransmitters. We will also attempt to influence absence generation by pharmacologic challenges of catecholamines.

B. Specific animal projects

1. Experimental models of generalized seizures of the absence type

Over the course of the last 15 years, staff now in the LPP have been interested in the development of techniques for simulating the generalized seizure patterns, as well as the behavioral accompaniments, of absence epilepsy. The tradition of this type of model dates back to research by H. H. Jasper and colleagues (e.g. Hunter, Perot, Ingvar, Pollen, Weir) at the Montreal Neurological Institute beginning in the 1940's. These earlier studies depended for the most part on electrical stimulation of various brain regions, mostly in the midline structures of the diencephalon and mesencephalon. This work led to the concept of the "centrencephalic system" as described by Penfield and Jasper and antedated the "reticular formation" by some years. It also influenced the thinking of many brain scientists about the cerebral bases of attention and consciousness. The neural bases of these behavioral states is still obscure; however, we are still impressed by the potentially close relationship between the brain system involved in generalized seizure discharges and that which is responsible for the maintenance of consciousness. Animal models thus remain important investigative tools in this research effort. We have explored the use of electrical, chemical, and metabolic methods of inducing discharges. In our laboratories, we have used in past studies: electrical stimulation of subcortical and cortical structures, administration of chlorambucil and pentylenetetrazol, subcortical implantation of aluminum hydroxide cream, and application of conjugated estrogen symmetrically to forebrain cortical surfaces. Most recently, we have done some experiments with compounds which block metabolism of GABA (i.e., gamma-vinyl GABA) and produce the paradoxical effect of inducing symmetrical and synchronous SW-like activity in the EEG of the monkey.

Stimulation of the mesencephalic reticular formation (MRF) and related areas in the monkey produces absence-like behavioral symptoms. The MRF is known to have far-reaching ascending influences which are likely to be the basis of the absence symptoms. These ascending influences are poorly understood in the monkey since more accurate work with radioactive amino acids as tracers has not been done. We have therefore begun a collaborative study with Dr. Linda Porrino (LCM) of the diencephalic terminations of the MRF.

Such models of generalized seizure activity permit the testing of various hypotheses and provide an experimental tool for study of single unit activity (as an example) under conditions of seizure.

2. Analysis of cellular activity in the attention system within the monkey brain

The concept of an attention system within the brain has been alluded to in various portions of our annual report. One could adduce evidence for the viability of this concept from a number of approaches or lines of evidence: the effects of lesions and other neuropathological conditions in human subjects; experimental lesions or other manipulations of the brain in animal subjects trained on tasks requiring or designed to accentuate attentive behavior; anatomical-physiological considerations. All of these lines of evidence appear to

converge in suggesting that there is a system within the brain that is specialized for the attentional needs of the organism. And one way of characterizing it is to study the attributes of individual nerve cells in those brain regions that allegedly form part of the system. We have conducted studies of two such brain regions to date: the meso-ponto-diencephalic portions of the brainstem and the prefrontal cortex, including both lateral and medial portions. Both of these locations contain large numbers of a particular type of cell which appears to have a specialized function with respect to attention. Our method of identifying this cell type has been based upon an experimental test paradigm in which animals are trained to perform in a go, no-go visual discrimination task. Attention-related cells are those which fire in both go and no-go trials. That is, they fire on trials when the animal has merely to attend to a stimulus display and performs no obvious motor response except holding a paw on a response key (no-go trials); they fire as well on trials on which a brisk lift and hit response to obtain a fruit juice reward is necessary. The cellular response may be identical on both trial types and may in addition anticipate the onset of the cue stimulus by several hundred milliseconds. This type of cell we have labeled as type II and, as noted above, is found distributed throughout certain regions of the brain stem and prefrontal cortex. Further studies will be aimed at providing a systematic functional analysis of this cell type, searching for other loci in the brain where they may be found, characterizing the relation among the several loci, and beginning pharmacological studies of these cells. As will be documented more fully in a project report below (Z01 MH 00506-03 LPP), a gradient of type II through possibly transitional cells through type I cells has been described in certain regions of the monkey frontal lobe. This work suggests that there may exist in the premotor area of the monkey a transitional cell type between the "attention" cell and the motor execution cell. This cell type may serve some bridging or connecting function between the attentional and motor execution systems.

3. Brain lesion and state change effects on visual attention

There is collaboration with LN and LCM in some parts of this work. One of the goals has been to specify the role of non-visual regions of the primate cerebrum in visual attention, and this work has used several techniques including lesions, stimulation, and electrographic recording of brain potentials under various lesion and non-lesion conditions. Other studies have been concerned with the recording and analysis of visually-evoked potentials under a variety of perceptual conditions, and with drug or sleep state effects on the mechanisms of visual attention.

C. Psychobiology of cognition

The aim of this research has been to relate psychological and biological determinants of various components of cognition that are involved in the acquisition, processing, retention, consolidation, and retrieval of information or experience.

Experiments have been designed to characterize and contrast cognitive failures in patients with disturbances of mood, children with various forms of impairment in learning, and populations of neuropsychiatric patients including those with Huntington's disease, Korsakoff's psychosis, and Alzheimer's disease. How are specific forms of central nervous system changes related to discrete alterations

in cognitive processes? Components of information processing in unimpaired individuals are also studied. A third type of study of both unimpaired individuals and patient groups uses various types of drug manipulation to alter specific aspects of cognitive processes. How do drug treatments that alter the activity of discrete aggregates of neurons relate to qualitatively different changes in cognitive processes?

1. Studies of depression

We can now define with some precision the kinds of cognitive processes that are impaired as well as those that are left relatively intact in patients with disturbances in mood (depression). Depressed patients are impaired in some but by no means all aspects of learning, memory, and retrieval. In part, this is a function of the intensity of their depression; moreover, the retrieval of experience is in part mood state-dependent. However, some aspects of information processing are particularly likely to be interrupted. Using models that distinguish between automatic versus effortful (or passive versus active) processing in cognition, we have noted that depressed patients may be profoundly impaired when cognitive processes require active or effortful as opposed to passive or automatic kinds of operations. For the latter, the depressed patient may be indistinguishable from a normal control. In fact, the extent to which depressed patients are able to manifest effort is highly correlated with the intensity of their depression and with the extent to which they can perform these non-automatic active cognitive operations. Also, the extent to which depressed patients are provided with organization of structure (as opposed to random uses) in information-processing tasks is the extent to which they are relatively indistinguishable from normal controls in terms of learning and memory.

How depressed patients think about information appears to determine many aspects of their problems in learning and remembering information. In concept learning, depressed patients develop overgeneralized hypotheses and do not "appreciate" disconfirming feedback. Their thinking appears to be global or overgeneralized and therefore, their processing of new information results in the formation of poorly elaborated weak memory traces. This can also be seen in how they process emotionally salient vs. "neutral" information and other stimulus characteristics that should ordinarily lead to effective information processing.

Recent studies in patients with Parkinson's Disease (PD) further highlight the distinction between impairments in automatic and effortful cognitive operations. Early stage PD patients experience memory-learning failures on tasks that require effort but not those that can be accomplished automatically. The memory-learning pathology is very much like that evident in depressed patients and unlike that seen in progressive dementia patients of an Alzheimer's type. We have also been able to show that mood changes along with changes in personality configurations (as seen in multiple personality syndrome) affect changes in how experiences are encoded and organized and also how they are retrieved from memory. Those cognitive changes are similar to drug related state-dependent learning and state-dependent retrieval effects.

2. Studies of dementia

Patients with progressive idiopathic dementia (most probably of the

Alzheimer's type) have classically been diagnosed on the basis of the cognitive state that they present clinically. These patients demonstrate profound impairments in learning and memory as well as other kinds of cognitive operations. We have now described more fully the characteristics of the cognitive dysfunction in these patients and defined some of the mechanisms that determine their learning/memory failure. In a study of a large series of early stage Alzheimer's patients, we have demonstrated that information is lost from memory relatively rapidly, that immediate memory is relatively unimpaired, and that any type of learning/memory operation that requires the establishment of permanent trace events is dramatically disrupted. These impairments are due in large part to processing or acquisition deficits which then result in weak trace formation and, therefore, failures to remember. Cognitive theorists have argued for a distinction between semantic memory (that is, the repository of information about how knowledge is organized or structured) vs. episodic memory, or the memory for ongoing, recent events. Although these two kinds of memory have been viewed traditionally as being separate and distinct, we have found that there is an important linkage between the two, one that defines in large part the characteristics of the memory impairment in Alzheimer's patients. We have shown that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results can have important implications both diagnostically, in distinguishing this group of patients from other groups, as well as for potential treatment strategies.

Cholinergic drug treatment of progressive dementia patients has proved to produce weak enhancement of some components of information processing. It would appear that those patients who are least cognitively impaired are most likely to benefit from such treatment. In contrast, vasopressin-treated progressive dementia patients have shown temporary marked changes in learning and memory, particularly in those subjects who can access previous knowledge better, following such drug treatment.

The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies to distinguish between these two groups clinically. Three dimensions that are particularly useful in distinguishing the cognitive impairment in depression from that of the progressive idiopathic dementia patient are: (1) that of automatic vs. effortful processing; (2) the degree to which effort is extended in accomplishing tasks; and (3) the extent to which the information being processed is either random or highly structured.

3. Learning disabilities

We have examined cognitive changes in children with various forms of learning disability. Two groups of these children have been studied: in one, hyperactivity is part of the syndrome; in the second, there is no evidence of hyperactivity or generalized retardation. All of these children demonstrated dramatic impairments in learning and memory that resemble the kinds of cognitive losses seen in some groups of adults. The resemblance is closest to depressed patients, but it also mimics the kinds of cognitive changes that are produced by drugs which disrupt cholinergic and noradrenergic activity. Thus, both hyperactive and learning-disabled children demonstrate impairments in effortful

processing of information, whereas automatic processing is left relatively intact. In fact, on our incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children are also impaired in those characteristics of cognition that require the imposition of organization in memory.

4. Pharmacological studies of cognition

Recent studies have shown that the dopamine system is involved in those types of cognitive operations that require effort but not for cognitive processes that can be accomplished automatically. When elderly unimpaired subjects were treated with L-Dopa/carbidopa their recall of information was enhanced (effortful processes) but their memory for how often an event took place (automatic processing) was unaffected. This apparent specific role of the dopamine system in modulating some types of cognitive processes is important both for what it teaches us about the psychobiology of cognition and its potential for the development of therapeutic strategies that would reverse cognitive impairments.

We have also begun to explore the role of the serotonin system in cognition. We have been able to show that zimelidine, a relatively specific 5HT reuptake blocker, can substantially reverse the cognitive impairing effects of acute doses of ethanol in man. Ethanol disrupts the memory for weakly processed information and zimelidine reverses this acute amnesic-like effect. It would appear that the serotonin system may serve to amplify weak memory traces.

In both patient groups and unimpaired subjects, we have found that cholinergic antagonists and agonists produce cognitive effects that are qualitatively different from those of drugs that have their major action on catecholamine activity (either as agonists or antagonists). A series of studies has been completed demonstrating the role of neuropeptides, such as synthetic vasopressin-like substances, in enhancing aspects of learning and memory. The enhancement occurs not only in cognitively impaired depressed patients but in unimpaired subjects and in patients with progressive idiopathic dementia as well. The general pattern of these findings implies that different neurotransmitter systems and/or neurochemical mediators may be involved in the regulation of various aspects of the acquisition, retention, and retrieval of information.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that: (a) cholinergic antagonists, such as scopolamine, mimic many of the characteristics of progressive idiopathic dementia; and (b) combinations of cholinergic agents produce small but reliable enhancements of some aspects of learning and memory in such patients. The amount of enhancement in learning and memory is limited by the degree of cognitive intactness of the patients. While alcohol has been viewed traditionally as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory (in collaboration with NIAAA) has demonstrated that post-processing manipulation, including treatment with alcohol, can produce some enhancement in learning and memory. This paradoxical improvement, as well as that evident in the effect of vasopressin on reversing retrograde amnesia following administration of ECT, implies that it is important to continue to explore the biological events that succeed information processing.

D. Autonomic nervous system activity in attention and psychopathology

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, and (4) the neurobiological basis of attention and ANS functioning.

In previous research we found that a pattern of poor attention as indexed by slow reaction time (RT), high ANS activity, slow adaptation and habituation, and a sluggish ANS response to stress were associated with a schizophrenic diagnosis and poor prognosis. In ongoing analyses of a more recent study, we have confirmed most of these findings for the schizophrenic group as a whole compared to controls. Further analyses with respect to clinical ratings and biological markers are underway. Patients with large cortical fissures and sulci differed from patients with normal CT scans in showing low ANS reactivity to novel stimuli and situations and slow recovery from stress. This may be related to low dopamine activity in this group as revealed by CSF studies, but since the ANS results for the normal CT patients differed from control values in the same direction, the data suggest a quantitative rather than a qualitative difference between groups contrary to speculations that have been advanced elsewhere. In a current study on schizophrenia, a major aim is to compare several RT methods of assessing attention deficits, some old and some new to this study, in an attempt to define more precisely the nature of attention impairments in schizophrenia and to investigate hypotheses about the role of ANS activity in attention impairments. These methods have been used in two studies on nonpatient males selected on the basis of extremely good or poor scores on either a continuous performance task (CPT) or pendulum eyetracking performance, two tests on which schizophrenics show marked impairments. Poor CPT subjects showed increasing RT impairment with uncertainty about the modality of the stimulus (visual or auditory). Subjects with poor eye tracking were impaired most when there was uncertainty about the time of the onset of the critical stimulus. This suggests that eyetracking and the CPT are tapping different aspects of attention.

Diagnostic specificity of the measures is being investigated by testing patients with a major depressive illness, patients with severe obsessive-compulsive neuroses, patients with panic-anxiety disorder and adults with a history of infantile autism as part of the same protocol being used with schizophrenics.

In a previous study, many of the variables which predicted clinical outcome in schizophrenics were independent of clinical state, suggesting that they may represent traits. In addition, in a previous study from this laboratory on twins, many of these variables showed a significant genetic influence. State influences are being studied in two experiments. In one, the subjects are patients diagnosed as multiple personality. Nine patients and two controls have been tested in four to five sessions in a design which enables comparison of within personality with between personality variations for each subject. Results to date show that a majority of the subjects exhibited significant between personality variations in RT. Analyses of ANS data are in progress. In another ongoing study, women are tested in the premenstrual and follicular phases of their menstrual cycle. Some

of the women are selected on the basis of regularly developing severe affective symptoms during the premenstrual period. This study may allow us to develop ANS markers for affective states. In a third study related to state influences on ANS recording during rest, a series of simple tones and a two-flash discrimination procedure are done on normal volunteers when they are either supine or standing. It is known that this postural change doubles the level of plasma norepinephrine. Preliminary results confirm previous studies in showing elevated electrodermal and heart rate base levels due to standing. In addition, ANS orienting responses to the tones increased in frequency and amplitude under this condition. Effects on two-flash threshold, a topic which has received considerable attention in the schizophrenia literature, differed for subjects scoring high or low on a paper-and-pencil scale of "psychoticism". The results suggest that this procedure produces increases in ANS activity, without confounded changes in attention or distraction, and that it could have wider application in testing hypotheses about the effects of "arousal" on behavior.

Neurobiological bases of attention and ANS activity are studied by investigation of the effects of drugs with fairly specific actions. Analyses will begin shortly on the comparative effects of several drugs on schizophrenia including pimozide, a dopamine receptor blocker, propranolol, a beta receptor blocker, and prazosine, a norepinephrine antagonist. A recent study compared the effects of an MAO type A inhibitor (clorgyline) with clomipramine, a tricyclic antidepressant, in obsessive-compulsive patients. Although only clomipramine was clinically effective, both drugs had similar effects in reducing electrodermal base levels, increasing heart rate, and reducing heart rate variability. However, clomipramine had stronger effects on heart rate and its variability, and only this drug reduced ANS responding to simple tones and task related stimuli. This result is of interest because ANS hyperresponsivity has been implicated as an etiological factor in theories of obsessive compulsive neurosis. Moreover, the patients with the greatest clinical improvement showed the largest drug effects on several of these variables.

The ANS effects of a chronic (2-week) period of high dietary caffeine consumption vs. placebo in normal children is being investigated in collaboration with Dr. Judith Rapoport of LCS using a design that also permits testing the effects of habitual caffeine use. Previous work on this project has shown that acute effects of caffeine produce ANS changes similar to that seen in anxiety states so that this study may have implications for the pharmacology of anxiety as well as for public health. Since few, if any drugs have completely specific effects, a variety of treatments will have to be investigated to specify the neurobiological bases of the ANS variables.

E. Individual differences in the study of heredity and environment in schizophrenia

The project is composed of the following studies:

1. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome.

The first study of this family was completed and published in book form in

1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed, so far as data-gathering was concerned, in June of 1981.

This study includes a number of the same variables that were examined in the quadruplets in the late 50's (e.g., CPT, reaction time, autonomic nervous system arousal and habituation) and adds a considerable number of new methods and techniques for assessing behavioral and biological factors. Among the new methods are the CAT scan, the PETT scan, far-field brainstem auditory evoked potentials, cerebral mapping of various EEG components and measures of many biogenic compounds from blood, urine, and CSF whose existence was unknown or for which no link to psychopathology had been established twenty years ago. The focus of this effort remains the same as it was in 1963: to help illuminate the differences in the severity of illness among these women. We have added, where possible, studies of the mother of the quadruplets, of the husband of the one who has been married, and of the two sons who have issued from that marriage. This additional information may also shed some light on possible genetic factors in schizophrenia. The data have been analyzed fully and are under editorial review (three manuscripts).

2. Studies of adoptees with schizophrenic parents and their biological and adoptive families

This represents a completion of work done with a cohort of adoptees and controls that were obtained in Denmark. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. This is in the course of being completed at this time. In addition, a reanalysis of the original case material has been completed and will appear shortly in the American Journal of Psychiatry. Several other papers are in preparation.

3. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel

This study, begun in 1965, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents together with 50 matched controls. Half of each group were raised on a kibbutz, and half were raised with their nuclear families in cities and towns in Israel. The children have been seen twice: once in 1966-7, when they were about 10 years of age, and once in 1973, when they were about 17. Aside from several reports of the neurologic findings (suggesting more "soft" signs in the index cases) none of the work has been published. Major progress has been made this year: (1) A series of manuscripts have been prepared describing available aspects of the 1966 and 1973 studies. In addition, major portions of the data have been analyzed. These efforts will form the basis of a comprehensive report to be published in a single issue of the Schizophrenia Bulletin. (2) In 1981, a third study of the original Israeli cohort was begun, and has now been completed. The data are being analyzed. The results are startling, since they suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family within a city environment. A paper is being prepared on the 1981 followup study which is to be submitted to Science. Some of the results were presented at the 1983 meeting of the American Psychiatric Association.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

F. Cognitive, perceptual, and nosological studies in depression

This project has involved a variety of studies, of which six are still being completed. All are at the stage of final data collection and are also at or near the completion of data analysis. Progress over the past year will be summarized below, while more detailed descriptions appear in the individual project reports. Since these projects were under the direction of Dr. Edward Silberman, who left the LPP, no additional data are being collected. It is expected that all the projects will be concluded shortly. Dr. Silberman remains a Guest Worker in the laboratory.

1. Psychomotor and psychosensory symptoms in affective illness

Data have been collected on a total of 111 patients with affective illness, partial complex epilepsy, and hypertension (controls). Preliminary analyses indicate that affective and epileptic patients are indistinguishable in frequency of certain kinds of visual, auditory, visceral, and cognitive illusions and hallucination, while paroxysmal motor phenomena are present only in the epileptic group. The relationship of such symptomatology to underlying personality factors and life course of illness in the affective group is now being analyzed.

2. Cognitive processing of emotional and neutral verbal stimuli in depression

Depressed patients were found to discriminate between highly emotional and neutral stimuli in a manner similar to normal controls. However, memory performance demonstrated a different pattern of remembering of high vs. low emotional, and high vs. low concrete stimuli for the two groups. These differences have been interpreted as demonstrating relatively shallower processing of semantic properties of material by depressed subjects. A manuscript describing this work has been submitted for publication.

3. Hypothesis testing in depression

Depressed subjects have been found to perform poorly relative to normal controls in an abstract reasoning task necessitating formulation and testing of solution hypotheses. Two types of deficits, inability to sufficiently narrow down sets of possible problem solutions and perseveration on disconfirmed hypotheses both contributed significantly to the depressive performance deficit and distinguished depressed from normal subjects. While elementary functions of memory, attention, and logic appeared to be intact, these could not be coordinated efficiently in the context of problem solving in the depressed group. A manuscript has been submitted for publication.

4. Lateralized hemispheric function in depression

Depressed female subjects have been found to show right hemisphere advantage

in a verbal processing task which normally displays left hemisphere advantage. Since such a task is an obligatory left hemisphere task in normals, these results suggest shifts of hemispheric function as a concomitant of the depressed state. Further studies involving male depressed subjects and depressed-recovered patients are now being planned. A manuscript has been submitted for publication.

5. Relationship of cognitive deficits to diagnostic sub-type and neuroendocrine changes in depression

Twenty-seven depressed patients have been tested with a battery of learning and memory protocols. Degree of memory impairment was not related to presence vs. absence of DSM III melancholia, or to normal vs. abnormal TSH response to TRH challenge. However, while patients with normal cortisol suppression following dexamethasone administration displayed the usual depressive deficits, dexamethasone escapers did not. Cognitive performance was related to degree of hopelessness in the suppressor, but not the escaper group. While these preliminary results suggest that dexamethasone response may discriminate cognitively from non-cognitively related depressions, more data are needed before any firm conclusions can be drawn. This work continues at the Psychiatric Institute of Washington, D.C. with the collaboration of Dr. Steven Targum, formerly of NIMH. These results have been presented at the 1982 meeting of the American Psychiatric Association.

6. Atypicality in primary depressive illness

The results of a pilot study investigating life course of illness and biological measures in typical vs. atypical depressed patients have been published. In collaboration with Dr. Robert Post of the Biological Psychiatry Branch, NIMH, efforts are now under way to enlarge the original sample of subjects, develop prospective methods of quantifying atypicality, and investigating atypicality in relation to long-term outcome following treatment.

G. Clinical Infant Research Unit - Transition Report

The Clinical Infant Research Unit (CIRU) is currently completing a preventive intervention clinical service demonstration program for infants and their families, begun as part of the mission of the Division of Mental Health Service Programs. At the same time the CIRU is formulating a new research agenda in keeping with its new mission as part of the Laboratory of Psychology and Psychopathology, IRP. Because this unit is in transition, a brief narrative describing its background and new plans will be substituted for the traditional project report.

The program of the CIRU began over six years ago. In addition to offering preventive intervention services to multi-risk families and their new infants (beginning prenatally) assessment was made of a group of normal families and babies. The main questions and hypotheses were:

1. Would it be possible to identify reliably a range of psychopathologies quite early in infancy in both the infants and their families?
2. The patterns of psychopathology identified would include affective, cognitive and physical-neurological dimensions. It was assumed that intensive

clinical work (stemming from psychodynamic and developmental and structural perspectives--see Greenspan, 1979), including observing behavior in a therapeutic relationship, would reveal the most complete picture of the patterns of psychopathology in these infants and their families, the potential reversibility of these patterns and the techniques that on a case-by-case basis appeared useful.

3. The patterns observed would lend themselves to a new classification system of psychopathology in infancy and early childhood.

4. Case-by-case work with each infant and family would suggest the range of service system strategies and clinical techniques that would be required (e.g., the failures would teach as much as the successes). This approach would indicate, as well, which clinical techniques and provisions of service were most effective for the types of problems encountered.

5. Early forms of psychopathology would be partially or completely reversible, when access to the family and baby could be maintained and individually tailored clinical techniques used.

6. Severe infant and family patterns of psychopathology would not be reversed when access to the family was not possible for long periods of time.

7. When the "level of therapeutic relationship" achieved (using a therapeutic process scale) was controlled, there would be functional relationships between prenatal, perinatal and early developmental patterns as well as later developmental patterns in physical, cognitive and affective domains with the strongest relationships reflected in the affective functioning of the children and their families.

8. The level of therapeutic relationship attained would predict outcome at ages 3-5 more strongly than family or infant variables.

The CIRU worked with 50 multi-risk families and their infants, 20 teenage mothers and their infants and a small group of "normal" families and their babies. During the program a new instrument (the GLOS) was developed to assess reliable infant and caregiver affective adaptation and maladaptation. Patterns of psychopathology were identified in the infants as early as the first month of life (difficulties with homeostasis) and group differences between families receiving intensive intervention and those receiving evaluations, consultative crisis intervention and referrals to community agencies were also evident by 1 month of age. Interestingly, the "multi-risk" families who were recruited because of problems with child rearing were also found to be characterized by factors associated with a high degree of psychiatric disturbance. The literature usually describes such families according to attitudinal value, motivational and service usage problems; over 30 years ago it was estimated the multi-problem families constituted 6% of the population that used over 70% of the public mental health, social service, and health services.

Most important, however, were the findings from this study regarding the range of psychopathology that could be described in the early years of life. These included difficulties with using the senses for self regulation and taking an interest in the world, forming an emotional interest in the human world, learning "cause and effect" type affective signaling, and difficulties with developing, elaborating and differentiating symbolic or representational capacities. For each infant pattern we were also able to identify maladaptive caregiver patterns. These findings which are descriptive in nature and therefore difficult to present are briefly described in detail in our monograph,

"Psychopathology and Adaptation in Infancy and Early Childhood; Principles of Clinical Diagnosis and Preventive Intervention."

Equally important were the findings regarding the service system and clinical approaches that these families required, also described in the monograph mentioned above and in a new book of case studies entitled "Preventive Intervention with Infants and the Multi-Risk Family; Case Studies and Clinical Trends."

Analysis of the 3 to 5 year old outcomes are in progress. Preliminary trends suggest, however, that early psychopathology is highly reversible when there is continuing access to the families and that affective rather than cognitive patterns are both indicative of psychopathologic disturbances and more sensitive to preventive interventions.

1. Transition to a New Research Agenda

During this service demonstration effort to identify the earliest type of psychopathology and the preventive intervention approaches likely to be effective, we were struck by a number of unique patterns.

There was a group of babies that seemed to evidence early difficulty in processing information (i.e., they were apparently not able to use their senses effectively to regulate themselves or take an interest in various sights, sounds, and tactile sensations). This was evidenced by the lack of states of focused calm, alert attention. Many of them, if their caregiving environments were not exceptionally gifted, would go on evidence "attachment disorders." They would either look away rather than toward their caregivers and turn away from sounds or appear apathetic and unresponsive to a variety of social and affective gestures. While the caregiving environment, if chaotic or unresponsive enough, could produce a similar picture even in constitutionally sound infants, we were intrigued by these babies who evidenced difficulties in using their senses to regulate and attend and subsequently by 3-4 months of age evidenced a clear disorder of emotional and social functioning.

We found further that each of these babies were different. A few had the most difficulty with auditory stimuli while others evidenced difficulty with visual or tactile ones. We found that by providing "phase-specific experience" through their intact sensory modalities, while remediating the more vulnerable mode, we could facilitate recovery to age-appropriate emotional, social and cognitive patterns. This interest has led us to begin planning a research agenda focused on identifying early processing difficulties in infants as a basis for certain types of psychopathology. This research agenda is based on the developmental structuralist mold of psychopathology and adaptation developed from our prior studies (see Greenspan, 1979, 1981). The following brief overview will provide a picture of the hypotheses that may be considered. Specific project outlines will be developed in the near future.

2. The Sensory and Affective-Thematic Organizations

(a) This model suggests that both adaptation and psychopathology can be described by the way in which sensory and affective-thematic experiences are

organized. It may therefore be hypothesized that severely, clinically disturbed groups of babies and/or young will evidence disturbances in the organization of sensory processing and affective-thematic patterns.

(b) This model suggests that there is a relationship between the organization of sensory and affective-thematic experience. Therefore, it may be hypothesized that disturbances in affective-thematic experience will, if they are severe enough, be related to disturbances in sensory processing. Similarly, disturbances of sensory processing, if severe enough, will have an impact on thematic and affective organizations.

(c) Furthermore, this model would postulate that a highly adaptive organizational structure at a "higher" level may compensate for a less adaptive organizational structure at a "lower" level or vice versa. This would work as follows: Assume that a youngster has some mild difficulties in his early sensory organizations because of sensory processing difficulties. This leads to mild deviations in the early thematic-affective organizations (i.e., a youngster with difficulty in processing and modulating aggression and discharge of activity). Assume, however, that this youngster is in an unusually empathetic and sensitive environment which also set effective limits. This environment helps the child have confidence in and internalize fully his capacity for control, regulation, and modulation of impulses and affects. More importantly, it helps him develop a representational capacity wherein sensations and affects are labeled, especially in the affective-thematic areas that are vulnerable (i.e., aggressive feelings and discharge patterns) and can be transformed in accord with adaptive goals. Such a youngster, in spite of an early constitutional and maturational disorder in organizing sensory and sensory affective experience, may very well develop a highly adaptive personality structure because he has the capacity to perceive and interpret even usually overwhelming sensory-affective experiences. He therefore makes "sense" out of confusing messages. That such proclivities, when interpreted and transformed, may later contribute to creative or artistic endeavors in an interesting speculation.

Consider the other scenario. A youngster with similar processing difficulties and limitation in the early organization of affects and themes has a very confusing family milieu. He not only does not have an opportunity at the level of prerepresentational interaction patterns to learn to organize his early sensory-affect patterns, but at the representational level develops confused meanings (because the family interaction patterns are filled with anxiety, conflict, inconsistent and undermining experiences and/or regression to concrete modes of thinking when anxiety is present). In such a situation there would be a compromise organization of sensory-affect experience, presumably "sending" up confusing signals and messages for interpretation to the representational system which would have difficulty interpreting even clear sensory and sensory-affect patterns. The fact that such a youngster would look highly disturbed therefore, would not be surprising. He would be vulnerable at both levels, the early somatic and the later representational level.

Etiology and Prevention

A most important hypothesis arising from this model is based on the fact

that the developmental structuralist approach focuses on levels of organization of experience. Therefore no one sensory pathway or aspect of the early sensory, affective-thematic organization need be decisive in determining the capacity of the youngster to organize at relatively more adaptive levels. Even youngsters with rather marked sensory processing difficulties may be capable of achieving highly adaptive levels or organization. In such instances, however, even intuitive caregivers may need to be aided by a specialized preventive therapeutic approach. Based on this hypothesis it may be assumed that the critical issue is to provide the child with "phase-specific experiential nutriments" in whatever way possible so that the child can organize an appropriate level of experiential organization. In the case of deaf or blind children, for example, it is now well known that when the intact sensory modes were not used to provide the "phase-specific experiential nutriments," many of these children developed secondary sequelae and look either retarded or autistic. However, when the blind children's hearing, touch, and other modes are used to provide the phase-specific experiential nutriments, they developed relatively competently. Similarly, deaf children do much better when their vision is used to help them have access to a range of phase-expected experience (e.g., early signing).

It is therefore intriguing to consider that the same principle may apply for severe processing deficits in auditory, tactile, vestibular, or other sensory pathways. It is not far-fetched to assume that many of the severe emotional disorders, and even retardations, may involve a profound but highly specific disorder in the processing of information (including affective information). In this context it is interesting to note that studies of Downs Syndrome babies (Chicchetti and Sroufe, 1976, 1978; Butterworth and Chicchetti, 1978) suggests that selected processing problems may compound the overall cognitive deficits. For example, to the degree that the caregiving environment continues to overwhelm the vulnerable sensory mode, a youngster may experience the world as confusing. Secondary phenomena such as social withdrawal with severe gaze aversion, extensor rigidity, sensory motor delays, cross-sensory integration problems, may then result. Similarly, if the brain does not have access to "experiential nutriments" for structure building, cognitive capacities may fail to develop, not necessarily because maturation could not proceed, but because the information or the necessary opportunities for the youngster to practice his potential abilities are not present. In other words, the environment does not make available those specialized experiences which could foster age-expected levels of experiential organization through the intact sensory modes. Therefore it is constructive to speculate that if a processing disorder did not interfere initially with overall integrative functioning and did not involve all areas of cognition, but only one or even two sensory pathways (such as the auditory and tactile or auditory and vestibular), the prognosis might be quite good with appropriate patterns of care.

The above hypothesis leads to the preventive therapeutic principles of providing the phase-necessary information or "experiential nutriments" at whatever cost through the intact modes (in other words, circumventing the impaired mode; e.g., a severe auditory processing problem or severe difficulty of handling a special type of affect, and making the necessary experiences available to the other modes) while, at the same time, gradually remediating the impaired mode. This approach would be similar to the way one works with an

auditory sensory processing difficulty in an older child. With a baby the prognosis might be significantly better because of the brain's greater plasticity in the early years.

Perhaps the major challenge in testing the above hypothesis is the development of methods to assess affective-thematic and sensory organizations in distinct clinical populations. We have developed an approach to the former (the GLOS), a system of quantifying "indicator behaviors" representing clinically relevant features of the infant, caregiver and their interaction patterns at each developmental structuralist organizational level. A description of the system, scoring manual, reliability studies and rules of interpretation are not available (Greenspan and Lieberman, 1980; Greenspan, Lieberman and Poisson, 1983). Studies and reports of the psychometric properties of the GLOS instrument on approximately 800 dyads are in progress.

While developing methods to assess the integrity and organization of the infants' sensory processing capacities is obviously a difficult challenge, there are some promising approaches that merit brief comment. The reliance on the voluntary motor performance in relationship to a task which involves sensory discrimination has limitations because early in infancy there is variability of motor development which often may be independent of sensory processing abilities. Yet there are promising techniques emerging where the motor task from which sensory processing may be inferred is of minimal complexity, such as sucking behavior or visual preference behavior (Lipsitt, 1966; Caron, 1982).

Another "output" from which sensory processing capacities may be inferred involves using the autonomic nervous system as the "output" behavior and employing a time series "stimulus interrupt" model (Campbell and Stanley, 1966). By using time-series models, such as the Box and Jenkins ARIMA models (Box and Jenkins, 1976), it is possible to detect significant deviations from baseline activity for a given autonomic variable. (This involves using auto-regression techniques to model the baseline, use the baseline model to forecast into the future when the stimulus is to be presented, and then set up confidence intervals around the forecasted value to see if a deviation from the baseline due to the experimental stimulus is statistically significant.) This method would lend itself to study a stimulus challenge in each sensory system with any autonomic variable as an "output." Such studies could range from the evaluation of whether an infant has detected and/or habituated to a stimulus, to studies of expectancy and discrimination (where the stimulus configurations presented gradually become more complex). In addition, the stimuli could involve affective-animate as well as inanimate types. In selecting an autonomic "output" variable, there may be an advantage to selecting one from the parasympathetic nervous system because of the shorter response latency and greater specificity of this system. In this regard, the measures of heart rate variability at the respiratory frequency developed by Porges (1983, in press) are very promising. It would be useful, in addition to assessing each sensory pathway, to be able to evaluate sensory integration at the brainstem level. Here an extremely promising approach, a "continuity model," where a component of peripheral physiologic activity is presumably a reflection of central nervous system regulatory capacity has been developed by Porges (1983). Individual differences in rhythmic modulation of heart rate appear to be a sensitive index of vagal control of the heart (Porges, in press). In applied studies,

differences in rhythmic modulation of heart rate differentiates healthy infants from infants with a compromised nervous system (Porges, 1983) and relates to the developmental course of cognitive processes (Fox and Porges, in press). Adjunctive methods, such as brainstem potentials, could evaluate neural transmission of auditory stimuli (Despland & Galambos, 1980). In addition, the "coupling" between respiratory and heart rate patterns may also have potential as an individual difference measure of an aspect of C.N.S. integration (Porges, et al., 1981). Studies of early processing limitations may also profitably include a search for biochemical correlates of specific sensory and affective thematic organizational limitations. We would expect that particular processing disorders may provide greater specificity for partitioning clinical syndromes than the symptom clusters often used in later childhood and adulthood. The latter may constitute global secondary or tertiary sequelae of a primary defect.

In order to apply and refine methods to detect early limitations in sensory and affective-thematic organizations, the initial selection of the clinical populations is quite important. Often in the study of developmental psychopathology clinical populations are selected, not on the basis of psychopathology, but on a global developmental risk factor for cognitive or neuromotor lags. We would suggest, therefore, that in the selection of initial subjects, an unmistakable psychopathology in infancy be present. For example, two groups that would qualify would be infants with two or more months of severe aversive reactions to the human world and infants with two or more months of an extreme state and affective lability with chronic panic reactions and clinging behavior, may be intriguing to study to look for similarities in sensory-processing and thematic-affective organization deficits and limitations.

As the underlying mechanisms for processing animate and inanimate information are identified, it would be possible to study with greater specificity the traditional "high-risk" groups such as small-for-gestational age babies, low birth weight babies, and offspring of schizophrenics and manic depressives. In such studies it would be critical however to control for the relative adaptiveness of the caregivers since the "experiential milieu" may have the potential to ameliorate or intensify the early disorders.

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ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
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This has been a year of long-planned and long-looked-forward-to transition in the Laboratory's research program. By the end of Fiscal Year 1982, we had completed the manuscript of a volume reporting two decades of research on the interrelationship of social stratification, job conditions, and adult psychological functioning. [Work and Personality: An Inquiry into the Impact of Social Stratification, by Melvin L. Kohn and Carmi Schooler, with the collaboration of Joanne Miller, Karen A. Miller, Carrie Schoenbach, and Ronald Schoenberg. Norwood, N.J.: Ablex Publishing Corp., 1983.] This year required considerable further investment of time in the actual production of the book -- proof-reading, editing, preparation of a comprehensive, analytic index -- but the main intellectual work on the U.S. analyses of job conditions and adult psychological functioning had been completed. There remains considerable further work on cross-national validation of the American findings and on testing whether our overall findings apply to theoretically pertinent subgroups of the population. (For example, is the relationship between job conditions and psychological functioning similar for people at differing stages of career and life course?) Nevertheless, the main thrust of our research has shifted from the effects of social structure on adult psychological functioning to the effects of social structure -- including, but not limited to, those that are transmitted through the family -- on the psychological development of children.

During this past year, our major research efforts have been addressed to the following activities: a reconceptualization of the processes by which values are transmitted from one generation to the next; a major process of "tooling up" for analyses to come (mainly in the development of new indices); and two principal lines of data-analysis on issues pertinent to the psychological development of children -- one on the relationship between educational experience and children's personality development, the other on the transmission of values in the family. In addition, there have been continuing cross-national comparative analyses, for Poland and Japan, of the relationships between job conditions and adult psychological functioning; a newly-initiated study of the work experience of the deinstitutionalized mentally ill; and continuing methodological inquiry. This report will summarize all of these activities. It will also briefly summarize a general interpretation of the psychological consequences of environmental complexity, developed by Carmi Schooler out of the analyses he has carried out in the course of this research.

RECONCEPTUALIZING THE INTERGENERATIONAL TRANSMISSION OF VALUES

Our strategy has been to redefine a classic sociological problem -- the transmission of values in the family -- by treating value-transmission in its larger social-structural context. We see the transmission of values in the family as part of a much more general process by which social structure affects children's values and orientations, with the family as only one -- albeit a crucial -- institutional mechanism in this process.

By values we mean conceptions of the desirable -- criteria of preference. By parental values, we mean the values that parents would most like to see embodied in their children's behavior -- the characteristics they consider most desirable to inculcate in their children. Parent-to-child value transmission implies, at minimum, that children come to hold the same fundamental values for themselves that their parents think desirable for them. Most research on the transmission of values in the family has focused on the magnitude of the correlation between parents' and children's values rather than on the process of value-transmission within the family or on the part that the family plays in the larger process of socializing the child in society. Most investigators seem to have begun with the assumption that there would be a high correlation between parents' and children's values and have been surprised to find correlations on the order of 0.15 to 0.25 -- much lower than they had expected. But past studies have generally dealt with valuation of quite specific characteristics -- for example, honesty or obedience -- rather than with fundamental dimensions of values -- such as valuation of self-direction versus conformity to external authority. Moreover, those studies have not taken unreliability of measurement into account. Our analyses, to be discussed below, do take measurement error into account and do find substantially higher parent-child correlations.

The much more important issue, though, is that even a very high correlation between parents' and children's values would not necessarily mean that parents' values have been transmitted to their children. We have every reason to believe that the socialization process is more complex than that. Parent-to-child value transmission is embedded in a larger social context, a context that is to some extent deliberately designed by parents but is to some extent beyond parental control, often beyond parental knowledge. Moreover, while it is true that parents and children experience largely similar social environments, it is also true that their environments diverge in significant ways. Not only do parents and children spend much of their time doing different things in different institutions, but the same social-structural conditions may impinge on adults and children in decidedly different ways.

Such complex processes are not going to be understood just by asking, "What is the zero-order correlation between parents' and children's values?" or even, "What is the partial correlation between parents' and children's values, statistically controlling social-stratification position, sex of parent, sex of child, and whatever else seems relevant?"

On the contrary, it will be necessary to develop causal models in which pertinent variables will not be "statistically controlled" in the partial-correlational sense, but in which their direct and indirect influences will be assessed as part of an ongoing process. We should ask how parent-to-child value transmission fits into a much more general process whereby a child's conditions of life, both those that are intentionally and those that are inadvertently imposed by parents, affect that child's values. This perspective can be made more explicit by a preliminary and highly schematic structural model of the process of value transmission (see the Figure).

In this model, parental values are not treated as the beginning of the process, but are seen as intervening in a chain that starts with the social-structural conditions of parents' lives, particularly (but certainly not only) their positions in the hierarchical ordering of the society. This is the part of the model on which we have concentrated most of our efforts thus far. Carmi Schooler's, Carrie Schoenbach's and Melvin Kohn's analyses of longitudinal data for the United States, and Kazimierz Slomczynski's, Joanne Miller's, and Melvin Kohn's comparative analyses of data for Poland and the United States, provide firm evidence about the causal interrelationships of education, occupational position, job conditions, and parental values. In particular, we know that both education and the job conditions that facilitate or inhibit the exercise of occupational self-direction -- namely, the substantive complexity of work, routinization, and closeness of supervision -- have a decided impact on parental valuation of self-direction versus conformity to external authority. We have also found that parents' values, in turn, affect parents' job conditions -- presumably because parents' values for their children reflect their more general values, for themselves as well as for their children, and people's values do affect how they perform their jobs. The relationships between occupational self-direction and parental values are therefore shown in the Figure to be reciprocal.

The second issue is so obvious as to be embarrassing to raise, yet it has received surprisingly little attention in the research literature: Mothers and fathers often do not have the same values. Our U.S. data show, for example, that the "true" correlation between mothers' and fathers' valuation of self-direction versus conformity to external authority, for children of both sexes aged 13-25 years, is approximately 0.52. Although substantial, a correlation of 0.52 hardly constitutes perfect agreement. When parents' values differ, whose values are transmitted to the child? Does it depend on sex of parent vis-a-vis sex of child? Or does it depend on whether the child is closer to the mother or to the father? Or on other intra-family processes? Or on the social-stratification position of the family or its ethnicity? Even listing these possibilities makes it obvious that a single correlation between parents' and children's values is at best a crude average of possibly large correlations between some parent-child pairs and probably negligible correlations between other parent-child pairs. The schematic model in the Figure does not take all these possibilities into account. It does, at least, depict mothers' and

fathers' values as independently derived, albeit correlated, and as independently affecting children's values.

Third, despite decades of research in developmental psychology and in the sociology of socialization, all too little is known about the processes by which parents' values are communicated to their offspring. Presumably, children infer parental values not only from parents' assertions but also from their practices. In all probability, disciplinary practices are involved, insofar as parents' values guide their disciplinary practices (as some of Kohn's earlier research showed) and these disciplinary practices exemplify for children their parents' values in action. Presumably, too, by affecting parents' more general conceptions of their responsibilities vis-a-vis their children (as some of Kohn's earlier research also showed), parental values affect the nature and quality of the parent-child relationship and, in this way too, exemplify for children their parents' values. But the very generality of these statements -- prefaced as they are by "presumably's" -- is an admission of how little is actually known. We don't even know much about the accuracy of children's perceptions of their parents' values. Nor do we know whether accurate perception of parental values is necessary for a child's coming to share the parents' values -- which is why, in the Figure, we treat children's perceptions of their parents' values as a possible but not as a necessary intervening variable from parents' to children's values.

Fourth, we shall have to conceptualize the ways in which children's outside-the-family worlds are structured. The crucial questions to ask are: "What are the immediately impinging conditions of a child's life?" and "How are these conditions socially structured?" We consider it necessary, for example, to analyze children's work in school much as we analyze adults' work in factories and offices -- in terms of its degree of substantive complexity, closeness of supervision, and the like. Karen A. Miller, Carmi Schooler, and Melvin Kohn are presently engaged in just such research. This research is discussed in some detail below.

For now, the Figure merely depicts "educational self-direction" as one of the "social conditions of the child's life," without showing its social-structural sources. A more complete model would have to trace such links as those between parental social-stratification position, parental values, public or private schooling, school curriculum (including the critical issue of school track), and educational self-direction. Our treatment of other social conditions of the child's life is even more nebulous: We merely assign to a black box "the influences of siblings, peers, and neighborhood." In so doing, we acknowledge but do not really deal with the existence of that large, albeit inconclusive, literature on possible conflicts between parents' and peers' values, leaving the entire question, as well as the associated question of the social-structural sources of peers' values, out of our schema. The Figure doesn't even note the influence of television, an influence that the NIMH has spent several million dollars studying. Our formulation is admittedly primitive just where we think new conceptualization is most urgently needed, in

understanding the factors outside the parent-child relationship that affect a child's values.

Fifth, despite the depiction of the Figure, we cannot assume that a socialization model is necessarily additive. On the contrary, we should start with the hypothesis that the central processes are interactive, in the analysis-of-variance sense of interactive. We hypothesize, for example, that the closeness of parent-child relationships will prove to be important in affecting children's values, not because a close parent-child relationship is itself conducive to the child's developing any particular value orientation, but because parental values will be more successfully "transmitted" to children by those parents who have close relationships with their children than by those parents who do not. Unfortunately for ease of research design, many of the components of the model may interact with other components. It may even be that parents' values will have greater or less effect on children's values depending on the type of school or neighborhood. Moreover, all of the components may affect children's values differentially, depending on the age and sex of a child -- a possibility much too complex to be shown in the Figure, but one that must be kept in mind throughout. Our schematic outline simply treats all relationships as if additive, it being too difficult to do otherwise in such a depiction. A systematic analysis, however, must test the possibilities of interaction at many places in the model.

Sixth is a question -- or really, a set of questions -- about timing. The model is structural, not dynamic; that is, it does not deal with rates of change in the transmission of values, only with total effects. Even so, questions of timing enter the model in several ways. There is, to begin, the question of when to measure children's values, which may change considerably during children's formative years. Should we expect children's values to be most like those of their parents when children are quite young and presumably most subject to their parents' influence? Or later, as children develop further? Or perhaps not until they, themselves, are adults? The issue has not yet been systematically considered or addressed empirically. Moreover, children's experiences may have different impacts on their personality development depending on the stage of the life course at which the experiences are encountered. Then, too, the same experiences may matter differentially in different historical contexts. In the schematic outline of the Figure, all that we have been able to include is one aspect of timing -- the question of whether it is parents' values when a child is young, as that child grows older, or some combination of the two, that affects a child's values.

Seventh, and finally, an adequate model of value transmission is made even more complex by the likelihood that children's values may not only be affected by, but may also affect, their parents' values. We have therefore shown these effects as reciprocal in the Figure. While our previous work shows that it is possible to assess reciprocal effects empirically, such assessment is difficult and may require exceptionally rich longitudinal data. The problem is made even more complex when we recognize that values may also affect and be affected by other

psychological phenomena, including intellectual functioning. This complexity we have not even dared indicate in the Figure, lest our "schematic outline of the transmission of values" become a grandiose model of the entire social psychology of the child.

In sum, the intergenerational transmission of values is a much more complex process than has generally been realized. Even calling the problem one of "transmitting" values may be so great an oversimplification as to be a misnomer. In particular, the transmission of values in the family is but one part, albeit a crucial part, of a much more general process by which a child's conditions of life in home, school, and in direct and vicarious experience of the larger social world come to shape that child's values. It is this process to which much of our current research is addressed.

TOOLING UP FOR NEW ANALYSES

A considerable amount of effort has been devoted this year to developing new indices for use in the intended analyses of social structure and children's personality development, as well as for analyses of social structure and personality more generally. Some of these efforts will be discussed below, in context of ongoing analyses of educational experience and children's psychological development and of the assessment of the correlations between parents' and children's values. In addition, Carmi Schooler has developed indices of intra-family process (from the parents' and children's data of our 1974 follow-up study, and from fathers' data in the initial 1964 baseline study of job conditions and personality), for use in our intended analyses of the role of parent-child relationships in the intergenerational transmission of values. Kazimierz Slomczynski has similarly developed indices of intra-family process from parents' and children's data of the Polish studies. Rather than describe these indices out of context, we shall leave their description to future Annual Reports, when we can describe not only how the indices were conceptualized and developed, but also how they were used in causal analysis.

Of somewhat more general utility has been the development of a method for disaggregating the components of social mobility. In studying the relationship between social mobility and psychological functioning, one of the crucial methodological problems is determining the components of social mobility. Kazimierz Slomczynski and Tadeusz Krauze (of Hofstra University) have provided a new tool for analyzing the components of social mobility. They showed that using the equation, "Circulation mobility equals total mobility minus structural mobility", in scalar rather than in matrix form, as has been done for more than two decades, limits the range of possible applications. Analyzing the generalized, matrix form of the equation, the investigators developed a satisfactory representation of traditional concepts of structural and circulation mobility fulfilling the criteria of face validity and consistency with formal postulates. They provided a method for decomposing the matrix of observed mobility (N) into a sum of three nonnegative matrices: immobility (I), structural mobility (S), and circulation mobility (C). In the future, the decomposition, $N = I + S + C$, can be used in analyzing the effects of the components of social

mobility on psychological functioning in terms of contextual variables for individuals.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The focus of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in our 1974 follow-up survey, when one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- then 13 to 25 years old -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions were designed to parallel those previously found to be useful for analyzing adult occupational experience, focusing on such dimensions of the educational experience as its substantive complexity and how closely the student is supervised. The intent is to see whether the concepts and methods developed for the study of occupational conditions can be applied as well to the study of educational conditions. The underlying hypothesis is that self-direction is important in young people's school experiences, just as in older people's job experiences. People who use initiative, thought and independent judgment in their daily work, whether in school or paid employment, will come to think more effectively not only in work but in other spheres of life. The psychological process is one of learning and generalization.

Opportunities for educational self-direction are, to a substantial degree, built into the structure of the educational system. The investigators assume (and test in their analysis) that there is a direct relationship between grade-level and the exercise of educational self-direction, with students at higher grade levels having both greater opportunity and greater need to exercise educational self-direction. Opportunities for educational self-direction also may be affected by other characteristics of the school and the educational system -- e.g., available economic resources, the school's educational philosophy, and the quality of the teachers. Moreover, the school staff may provide students differential opportunities for self-direction, giving apparently brighter students greater opportunities to do self-directed work and apparently less bright students fewer such opportunities. Finally, educational self-direction is not entirely determined by the properties of the school and the differential treatment of the students. Even though opportunities for educational self-direction are to a substantial degree built into the structure of the school, there is still some leeway for similarly situated students to differ in how they respond to these opportunities. Students with higher levels of cognitive functioning may maximize whatever opportunity the school affords to do self-directed work; those with lower levels of cognitive functioning may avoid opportunities for self-direction. The hypothesis is that educational self-direction may not only affect, but also be affected by, cognitive functioning.

In empirically assessing this hypothesis, the lack of longitudinal data poses a serious problem: Ideational flexibility at earlier times cannot be statistically controlled in assessing the impact of educational self-direction on current ideational flexibility, nor can earlier educational self-direction be controlled in assessing the impact of ideational flexibility on current educational self-direction. This analysis does benefit greatly, however, from our having information about the ideational flexibility of all of the fathers and most of the mothers. This means that, in assessing the impact of educational self-direction on the ideational flexibility of students, parental ideational flexibility can be statistically controlled. This makes it possible to statistically control genetic and, to some degree, family-experiential determinants of cognitive functioning. A model of the reciprocal effects of educational self-direction and ideational flexibility thus includes parental ideational flexibility, students' ages and grade levels, the extent to which school courses are compulsory or elective, and pertinent social characteristics of the students and their families.

The analysis shows that the degree of self-direction exercised by students in their schoolwork has a decided impact on their cognitive functioning and that cognitive functioning, in turn, has a decided impact on the exercise of self-direction in schoolwork. Specifically, educational self-direction and ideational flexibility have substantial, approximately equal, reciprocal effects. The impact of educational self-direction on ideational flexibility results mainly from the substantive complexity of students' schoolwork -- its scope, difficulty, and challenge. The effect of ideational flexibility on educational self-direction results in part from more intellectually flexible students exercising greater self-direction in their schoolwork, and in part from intellectually flexible students tending to be in somewhat higher grade levels than are their age-mates -- and schoolwork is more self-directed at higher grade levels. Insofar as they can be tested, these conclusions apply to both secondary-school and college students.

The reciprocal effects of educational self-direction and ideational flexibility operate in context of other important processes: Parental intellectual functioning and student's age affect students' ideational flexibility; and parental socio-economic status and students' grade levels affect the amount of self-direction exercised in schoolwork. It is striking, though, that even in competition with these genetic, developmental, and social-structural factors there is a substantial reciprocal relationship between the actual work that students do and their cognitive development.

This reciprocal process parallels what has previously been found for adults in the workplace. The present findings thus extend our knowledge of how work and personality affect one another in different institutional settings and at different stages of the life-course. In particular, they show that the experience of self-direction in work is important not only

for the psychological functioning of adults, whose work is paid employment, but also for children and young adults, whose work is schooling. These findings also tell us something specific about education: They clearly support those who argue that the "quality" of school experience does make a difference. The salient feature of schooling for students' cognitive development is the degree of self-direction they exercise in their schoolwork, particularly the substantive complexity of that schoolwork.

In addition to the analysis of cognitive functioning, Miller, Kohn, and Schoenbach have begun an analysis of the relationship of educational self-direction to other aspects of psychological functioning -- in particular, self-directedness of orientation and psychological distress. Using confirmatory factor analysis, they have developed measurement models of self-directedness of orientation and psychological distress for children, analogous to those earlier developed for adult men and women. These models summarize several important aspects of personality. Self-directedness and distress are both "second-order" concepts. Self-directedness of orientation is indicated by personally responsible criteria of morality and by relatively low levels of authoritarian conservatism, fatalism, and idea conformity. Psychological distress is indicated by high levels of self-deprecation and anxiety, and by low levels of self-confidence, trust, and idea conformity. Preliminary causal models have been tested relating educational self-direction to both facets of orientation. In the case of self-directedness of orientation, the pattern of reciprocal effects is similar to (although not quite as statistically robust as) that for cognitive functioning; that is, educational self-direction, particularly the substantive complexity of schoolwork, leads to a more self-directed orientation, and a more self-directed orientation leads to the exercise of greater self-direction in schoolwork. The investigators have also found, as expected, negative reciprocal relationships between the exercise of educational self-direction and psychological distress. The substantive complexity of the schoolwork is again the major factor affecting psychological distress -- in this case, decreasing the student's level of distress. But closeness of supervision by teachers also plays a powerful role, increasing the distress of the student being supervised.

ASSESSING THE CORRELATION BETWEEN PARENTS' AND CHILDREN'S VALUES

Kazimierz Slomczynski, Carrie Schoenbach, and Melvin Kohn are attempting, as a first step in the analysis of the process of value transmission, to reassess the magnitudes of the correlations between parents' and children's values. They are using data from representative samples of parents and children in the United States and Poland. To develop indices of values, they employ confirmatory factor analysis. Since confirmatory factor-analytic models enable one to separate the underlying constructs from errors (i.e., idiosyncratic variance) in their indicators, such models provide a basis for calculating the "true" correlations between parents' and children's values, shorn of measurement error. They have developed a model for parents' and children's values in the United States

and are in process of developing a similar model in Poland. In both countries, they focus on valuation of self-direction versus conformity to external authority. In these analyses, they build a single measurement model of mothers', fathers', and children's values, rather than three separate models. From these analyses, it appears that the correlations between parents' and children's valuation of self-direction/conformity are considerably stronger than past studies had led us to expect. For the United States, using longitudinal data and considering values for children across the entire age-range of 13 to 25 years of age, the correlation between fathers' and children's values is approximately 0.58 and that between mothers and children is approximately 0.55. The analysis for Poland is still in process; it uses cross-sectional data and focuses on children in the younger half of that age-range. Preliminary indications are that the correlations for Poland will be not much lower than those for the United States -- probably just about the same magnitude as for U.S. children of the same age-range (when the U.S. data are analyzed cross-sectionally).

These correlations are much more in accord with a priori expectations than were those found in past studies -- a tribute, we believe, to the power of confirmatory factor analysis. But, still, even correlations of 0.58 and 0.55 leave much to be explained, with parents' values "accounting for" less than half of the variance in children's values. Moreover, as indicated in the provisional model of value-transmission discussed above, calculating the correlations between parents' and children's values is only the first step in the causal analysis of the intergenerational transmission of values. The next step, on which the investigators will soon embark, is to develop causal models that assess such issues as the degree to which social stratification affects children's values through its effects on parents' values (then transmitted from parents to children) and the degree to which social stratification affects children's values (if it does) through other processes. Social stratification aside, the questions of the extent to which and the processes by which values are transmitted from parents to children can now be dealt with on a much firmer basis than before, since we now have much more realistic estimates of the actual correlations between parents' and children's values. It should also be possible in this research to assess the process of transmission of more general orientations than values, even to study the relationship between parents' and children's cognitive functioning.

THE POLISH REPLICATION

The main purpose of the Polish inquiry has been to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland,

Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed their analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? [The answers to both questions are positive with respect to values and social orientations, but not with respect to self-conception.]

Further analysis of the Polish data has taken three primary directions:

(1) The Polish investigators have carried out a new survey of the wives and children of a subsample of the men interviewed in the original study, with an emphasis on parent-child relationships and the psychological development of children. Slomczynski has been developing indices of family relationships and of various facets of mothers' and children's psychological functioning, and he, Carrie Schoenbach, and Melvin Kohn are utilizing these data for a comparative analysis of value transmission (discussed above).

(2) Joanne Miller, Slomczynski, and Kohn have extended the comparative study of job conditions and psychological functioning in the United States and Poland to focus on whether the impact of job conditions on personality is consistent for men at different stages of career and life-course. The analysis separately examines three groups of men: those 30 years or younger, those between the ages of 31 and 45, and those 46 years of age and older. The analysis is motivated by two central concerns: the possibility of changes in the malleability of personality at different stages of career or different stages of the life-course; and the possibility of particular experiences having differential impact, related to age. Age-related differences in the effects of experience on psychological functioning might occur because of biological aging, the proximity and duration of experience, or the differential significance of the same experience depending on stage of career. The international comparison tests the universality of consistency or discrepancy across age groups.

This analysis examines four aspects of personality: intellectual flexibility, authoritarian conservatism, self-deprecation, and self-confidence. All four facets of psychological functioning are included, because of differences in their stabilities (intellective dimensions of personality being most stable and aspects of self-concept least stable) and because they may be differentially responsive to job conditions at different stages of career. A highly stable characteristic such as intellectual flexibility may be maintained by a fairly constant and accumulating set of experiences, regardless of age, while an aspect of personality that is less stable, such as self-confidence, may be

differentially responsive to job conditions (and other experiences) at different ages. For example, the impact of close supervision on self-confidence may differ at different stages of career.

The analysis required extensive evaluation of indicators used to measure psychological functioning. When age-specific differences in indices are found, it is essential to assess statistically whether the indices nevertheless capture equivalent concepts. Otherwise, age-group comparisons could be interpreted as measurement artifacts. Although some age-specific differences in sets of patterned responses were identified, these analyses indicate that it is possible to create conceptually equivalent indices of psychological functioning for men of varying age.

The emphasis of the study is now on the social, particularly the occupational, determinants of psychological functioning. Specifically, the investigators are testing whether those conditions that facilitate or constrain opportunities for occupational self-direction have consistent effects for younger, middle-aged, and older men. These analyses have not been completed. The initial findings for intellectual flexibility, though, are intriguing. Consistent with a model that learning-generalization occurs throughout the life-course, occupational self-direction is found to affect intellectual flexibility (independently of pertinent social characteristics), regardless of the age of the worker, in both Poland and the United States. Particularly striking is the consistency and strength of the effect of substantive complexity on intellectual flexibility. Future analysis of other dimensions of psychological functioning will test the generalizability of these initial findings to other facets of psychological functioning.

(3) Recently three types of analyses have been undertaken to evaluate the reliability of the measurement model of occupational self-direction for Poland. Krystyna Janicka (of the Polish Academy of Sciences), Grazyna Kacprowicz (University of Warsaw), and Slomczynski started by studying the possible effects of variations in the procedures of data collection used in interviewing the 1978 national Polish sample. The focus of analysis was on the effects of the interviewers' characteristics (e.g., level of field experience) and modes of interviewing (e.g., number of probing questions employed), and the methods employed in coding the data (e.g., the extent of use of supplementary coding materials). None of these effects appeared to be statistically significant. Moreover, experiments of double and triple coding show a high reliability of each indicator of occupational self-direction; intercoding correlations are all more than 0.89, with an average of 0.92.

Further analysis has been based on data from a limited follow-up study. In this study, 100 men from the original 1978 national sample were interviewed for a second time with a schedule containing questions -- repeated verbatim -- about the substantive complexity of work. Two findings are important: the correlation between factor scores derived from the measurement models for the original and follow-up studies is very high

($r = 0.91$); and the hypothesis that measurement models for both studies are not based on the same sample is rejected at the .05 level of significance.

In addition, the structure of the model developed for the 1978 Polish data has been imposed on two independent sets of data: a 1972 national sample and a 1979 Warsaw sample. In all three studies, the estimates of the model are essentially the same for the substantive complexity of work, the most important component of occupational self-direction. Estimates for closeness of supervision and routinization of work have been analyzed only for the 1978 and 1979 data, because only in these studies were the indicators obtained through the same method. The inter-study differences in the estimates appear to be negligible. Generally, the similarity of the measurement models of occupational self-direction in different samples of the Polish population assures us that the reliability of the instrument for Poland is as good as for the United States.

THE JAPANESE REPLICATION

Another thoroughgoing replication of the Kohn-Schooler survey has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data collection took place during the summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed with a questionnaire containing all the questions necessary for indexing job conditions and those aspects of psychological functioning measured in the original study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Carmi Schooler. Confirmatory factor analysis was used to develop measurement models of occupational self-direction, intellectual flexibility, parental values, and several facets of self-conception and social orientation. These measurement models proved to be generally similar to those that had previously been developed for the American sample. Last year's Annual Report provided a detailed description of the causal analyses that Naoi and Schooler did with these data — analyses that generally confirm the U.S. findings.

This year's work on the Japanese study focused on the collection of new data and the preparation of both old and new data for further analysis. Michicko Naoi of the Tokyo Metropolitan Institute of Gerontology conducted a survey of the wives of the respondents in the earlier survey of Japanese men. Her questionnaire contains all of the key questions that had been asked of the wives in the American follow-up survey. We therefore now have the data to examine the generalizability to Japan of the U.S. findings about the psychological effects on women of the conditions of both paid work and housework. In addition to the questions about values for themselves and their children, from the American study, the Japanese women's questionnaire also contains questions about women's values and perceptions of their responsibilities vis-a-vis their own and their husbands' parents. These questions, when taken together with the questions the women were asked about the relative importance of their various roles and about the value they place on their household work and paid employment,

should permit the examination of the psychological effects of the web of obligations in which Japanese are said to be particularly enmeshed. These data have been coded and preliminary analyses begun.

Work also continues on the study of Japanese men. Data on jobs are being recoded to permit the examination of the effects of such theoretically important dimensions of work as whether it takes place in the primary or secondary sector of the economy and whether the occupation derives from traditional Japanese culture.

WORK EXPERIENCES AND THE DEINSTITUTIONALIZED MENTALLY ILL

An exploratory, participant-observer study of the relationship between work experience and recovery from mental illness is being conducted by Elliot Liebow, on detail to the Laboratory from the Extramural program. Field work began in March of this year. The data collection phase is expected to last through this calendar year.

The major data base will be derived from direct observation and personal interaction with men and women coming out of state mental hospitals and following them through their community experiences, focussing particularly on their work experiences and how these experiences affect the course of the disorder. The study population will be drawn from half-way houses, day treatment and vocational programs, and street people. Specifically, the research focuses on the way in which discrete aspects of the job (e.g., substantive complexity) interact with symptoms; how job characteristics and symptoms interact with social relationships and social functioning; and how all three systems -- work, symptoms, and social relationships -- interact with one another to affect the course of recovery from psychiatric disorder.

The goal of this exploratory research is not to test hypotheses but rather to grasp, so far as possible, the dynamics of work-symptom-social relationship interactive processes, and to use the hunches and insights that naturally flow out of first-hand, close-up observation over time, to generate hypotheses for a more systematic attack on the problem in the future. Early first impressions include: (1) There is much discussion about the importance of work among service providers and their deinstitutionalized clients but surprisingly little action; (2) the routine programmatic emphasis on "entry level" jobs tends to ignore the wide range of individual skills, strengths, and weaknesses of deinstitutionalized persons; and (3) many "entry level" jobs are among the most demanding, high-pressure jobs our society has to offer.

METHODOLOGICAL INQUIRY

The key analytical instrument of research in the Laboratory is a computer program written and maintained by Ronald Schoenberg called MILS (Multiple Indicator Linear Structural analysis). A new component was added to the program this year, to compute total and indirect effects and their

standard errors. This addition to the computer program has enabled considerable expansion of the analysis of causal relationships, heretofore restricted to the assessment of direct effects. Now the effect of an independent variable on a dependent variable through other variables can be assessed, and thus a relationship between these variables can be established even though a direct effect may not exist.

During the year, Schoenberg completed work on the extension of latent-variable methods to categorical and other limited variables -- i.e., variables measured in categories or measured in some way that precludes all possible numerical values. The new technique generalizes and elaborates upon current methods for the analysis of limited variables, such as logit, probit and Poisson regression. As a result, these types of models may now be incorporated into multivariate measurement models such as those employed in MILS. Extension of this work, now in progress, has important implications for data-analytic methods used throughout the social sciences. Schoenberg is also developing methods for testing the interaction effects of latent variables. This work will be crucial in the planned research in the Laboratory, since much of this research will require the examination of potential interaction effects (which occur when a basic relationship between two variables changes as a function of a third variable).

THE PSYCHOLOGICAL CONSEQUENCES OF ENVIRONMENTAL COMPLEXITY

In the course of his research, including his analyses of both occupational and non-occupational determinants of psychological functioning, Carmi Schooler has developed a general interpretation of the effects of environmental complexity on psychological functioning. According to this interpretation, the complexity of an individual's environment is defined by its stimulus and demand characteristics. The more diverse the stimuli, the greater the number of decisions required, the greater the number of considerations to be taken into account in making these decisions, and the more ill defined and apparently contradictory the contingencies, the more complex the environment. To the degree that the pattern of reinforcement within a complex environment rewards cognitive effort, individuals should be motivated to develop their intellectual capacities and to generalize the resulting cognitive processes to other situations.

Non-intellective aspects of psychological functioning may also be affected by environmental complexity. To the extent that complex environments reward initiative and independent judgment, such environments should foster a generalized orientation favoring self-directedness rather than conformity to external authority. On the other hand, values, orientations, and behaviors that are adaptive in complex environments may be maladaptive in simpler ones. Correspondingly, simple environments may not provide sufficient rewards to insure the development or continuance of relatively high levels of cognitive functioning and self-directedness. Consequently, continued exposure to relatively simple environments may result in a decrement in intellectual functioning and a change in values,

orientations, and behaviors in keeping with the level of environmental demand. If, as seems plausible, such adaptation can occur at any age, changes in environmental complexity should produce changes in intellectual functioning and related values and orientations at any point in an individual's life course: childhood, midlife, old age.

To assess the plausibility of this interpretation, Schooler reviewed evidence from a wide variety of sources, including research about environmental effects on children and the aged, as well as animal and social-psychological experiments. The review provides evidence that environmental complexity leads to more effective cognitive functioning at all stages of the life span. This effect has been found in both sexes, in several nations, and even in species other than man. Although the evidence is not as extensive, the review also indicates that environmental complexity leads to a self-directed rather than conformist orientation. Although none of the studies is perfect, and few are able to completely exclude alternate explanations, it is rare to find a theory, no matter how roughhewn, that is congruent with such a range of phenomena -- one that stretches not only across populations, but across the research of academic disciplines from biochemistry to sociology.

Proposed course of further research:

As is evident above, the analysis of the Polish and Japanese replications is incomplete, with much more to be done. The analysis of education, too, is far from complete. The analysis of the intergenerational transmission of values has barely begun. There are data in both the U.S. and Polish studies with which to carry out such an analysis. The investigators hope to elaborate and test the provisional model of the processes summarized in this report.

During this Fiscal Year, Melvin Kohn spent six weeks in Australia as a Visiting Fellow of the Research School of Social Sciences of the Australian National University, helping Jonathan Kelley develop a parallel study of the transmission of values in Australian families. This study has potential value for extending the range of our analyses, since the values to be studied go far beyond the range of values included in our own research. Kohn also spent three weeks in the Federal Republic of Germany, much of that time at the Max Planck Institut fur Bildungsforschung in Berlin, the rest visiting German universities. Preliminary plans were made for possible future comparative studies that may further expand the scope of the Laboratory's research. (There have been several studies in Germany that replicate parts of our United States-based research. Others are in progress.)

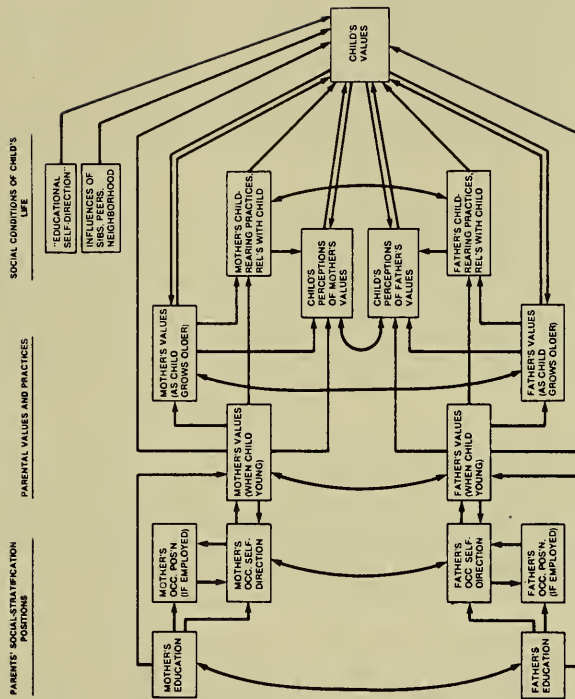


FIGURE 1. SCHEMATIC OUTLINE OF THE TRANSMISSION OF VALUES IN THE FAMILY.

Annual Report of Laboratory of Cell Biology
National Institute of Mental Health
Michael J. Brownstein, M.D., Ph.D., Chief
October 1, 1982 - September 30, 1983

INTRODUCTION

The Laboratory of Cell Biology was created in October, 1982. At present there are two tenured senior investigators who function as Unit Chiefs in the Laboratory: Drs. Brownstein and Zatz. In addition, Dr. Hirata (a Visiting Scientist who has applied for a permanent immigrant visa) heads a Unit, and Drs. Eiden and Pruss act as Unit Chiefs. Finally, Drs. Palkovits and Hökfelt (or their appointed surrogates) each plan to spend six months a year here, and they will jointly direct an anatomy group. In the long run, continuity will be provided by one or more permanent members in this group. I hope to "borrow" other scientists from their universities on a continuing basis. Joint appointments give us an opportunity to recruit new staff members who would not be available otherwise.

Work in the Laboratory of Cell Biology ranges from neuroanatomy to molecular biology. Neuroanatomical studies have been conducted by Drs. Palkovits, Hökfelt, Mezey, and their colleagues. These workers have used both light and EM immunocytochemistry to study diverse neuronal pathways in the CNS. In addition, the distribution of specific proteins (e.g., carboxypeptidase B, retinal G-protein) within neuronal elements has been determined. These ultrastructural studies (which depend on the availability of specific antibodies) provide insights into the roles of these proteins and the way in which cells are constructed.

Many of our projects involve purification and characterization of molecules. It is only by purifying molecules of biological interest that their functions and mechanisms of action can be unraveled. Examples of peptides and proteins that have been isolated are the salmon spawning factor and lipomodulin. Pineal serotonin N-acetyltransferase has been partially purified. New methylated lipids have been characterized, as well. (These have pointed at the existence of novel enzymatic reactions.)

Molecular biological techniques can be used to determine the structures of proteins. The amino acid sequence of preproenkephalin has been inferred from cDNA to its mRNA. The structures of the neuron specific enolase and of chromogranin A are currently being investigated. cDNA probes developed in the course of the above investigations should be useful for studying the regulation of protein production at the genomic level.

The development of the nervous system involves, among other things, the switching on and off of genes. By studying the expression of specific cellular products in cultured cells it may be possible to understand how neurons differentiate and how differentiated cells regulate the manufacture of the substances that they secrete. To this end, members of the Laboratory of Cell Biology have engaged in isolating molecules that are involved in the post-translational processing of peptide precursors, in the packaging of secretory proteins, in axonal transport of storage granules, etc. Monoclonal antibodies have proven to be powerful tools for isolating such molecules and establishing their roles in cells.

In addition to studies of development, members of the Laboratory of Cell Biology have been interested for some time in the phenomena of signal transduction and circadian periodicity. Biochemical studies of these two phenomena outlined below have begun to piece together mechanisms underlying them.

SPECIFIC PROJECTS

The structure of the salmon spawning factor was determined by Dr. Sherwood and her collaborators. This peptide, a relative of mammalian luteinizing hormone releasing hormone, is quite potent in inducing spawning and should have commercial applications. A similar, but not identical, molecule is being isolated from the brain of chickens.

Drs. Namboodiri, Klein, and Brownstein have continued their attempts to purify pineal serotonin N-acetyl-transferase. By combining affinity chromatographic methods and HPLC, this should prove possible. Having pure material represents the first step toward understanding the regulation of this enzyme's production.

Drs. Hook, Fricker, Snyder, and Brownstein have prepared polyclonal and monoclonal antibodies against the carboxypeptidase B-like peptide processing enzyme from pituitary. In addition, Dr. Hook has begun to characterize and purify the trypsin-like enkephalin processing enzyme from adrenal medulla.

Drs. Mezey, Reisine, Palkovits, and Axelrod have implicated peripheral catecholamines in regulating ACTH release from the anterior pituitary.

Drs. Mezey, Palkovits, Krieger, and Brownstein have shown immunocytochemically and by studying the effects of lesions on 5HT levels that serotonergic fibers in the intermediate lobe of the pituitary are provided by raphe neurons and hypothalamic neurons as well.

Drs. Mezey, Hamm, and Takahashi have localized G-protein to discrete populations of cells in the retina and central nervous system. Their light microscopic immunocytochemical studies are being extended to the EM level.

Drs. Mezey and Skirboll have identified cells in the hypothalamic paraventricular nucleus that project to medullary catecholaminergic nuclei. These cells do not appear to manufacture vasopressin or oxytocin, and Dr. Mezey hopes to identify the transmitter that they use by combining retrograde dye transport and immunocytochemistry.

Dr. Moskal has succeeded in preparing monoclonal antibodies against neurons in the dentate gyrus. These will be used in behavioral and physiological studies. Dr. Moskal is also making monoclonal antibodies against purified Golgi membranes and hopes to isolate antibodies against specific glycosylating enzymes.

Drs. Moskal, Baumgold, and Zimmerman have provided evidence for the existence of different molecular forms of the sodium channel protein in developing and adult rat brains.

Drs. Zamir, Palkovits, and Brownstein have examined the distribution of

the dynorphin family of peptides (dynorphin A₁₋₁₇, dynorphin A₁₋₈, dynorphin B, α -neoendorphin, β -neoendorphin, Leu-enkephalin) and certain members of the enkephalin family of peptides in the CNS. The data show that the dynorphin precursor may be processed differently in different terminal fields and that this precursor may be a major source of Leu-enkephalin in the brain. Dr. Zamir and his colleagues have also shown that the posterior pituitary is especially rich in dynorphin and that the source of most of this peptide is the supraoptic nucleus.

Dr. Russell has prepared monoclonal antibodies against calmodulin and calmodulin-related proteins; he is now making antibodies against secretory granules from posterior pituitary nerve endings. He hopes to use these to identify proteins important to the storage and release of peptide hormones.

Dr. Russell has identified two ATPases that are associated with secretory granules. One seems to be involved in axonal transport of these granules, the other with maintaining their ionic gradients.

Drs. Palkovits, Eskay, Eiden, Douglas, Beinfeld, Williams, and Kiss have used immunocytochemistry and lesion/microassay methods to characterize a number of peptidergic pathways in the CNS. They have paid special attention to the source of innervation of the median eminence and posterior pituitary.

Drs. Faden, Chiueh, Beinfeld, Zamir, Hökfelt, and Brownstein have examined the effect of TRH treatment on biochemical alterations that follow spinal cord trauma. While TRH protects against spastic quadriplegia, it does not seem to affect the loss of transmitters from descending neuronal pathways.

Drs. Brownstein, Marangos, Evans, and Rosenfeld are in the process of isolating cDNA to neuron specific enolase (NSE) mRNA. This should allow the structure of NSE to be determined and the regulation of its biosynthesis to be studied.

Dr. Zatz and his collaborators have uncovered a novel enzymatic process by which S-methyl acylthioesters are synthesized on the cytoplasmic side of plasma membranes. These thioesters, if formed in vivo, could act as methylthio donors.

Drs. Zatz and Butler have shown that pantethine, a metabolic precursor for coenzyme A, depletes cystinotic cells of cystine. Pantethine acts by being converted to cysteamine within the cells. Systemic cysteamine is poorly tolerated by patients with cystinosis. Pantethine, on the other hand, is tolerated well and may be a safe and effective alternative to cysteamine for treatment of cystinosis.

Drs. Zatz and O'Brien have found that bovine retinas incorporate palmitate into rhodopsin. They hope to establish whether this reaction is enzyme catalyzed and then determine its role in visual transduction.

Drs. Zatz and Eskin have shown that cyclic GMP mimics, and may mediate, the effects of light on the circadian oscillator in the eye of Aplysia.

Dr. Takahashi has demonstrated that monoclonal antibodies against the G-protein regulating phosphodiesterase in the retina also recognizes the protein

that regulates adenylate cyclase in the rat pineal.

Dr. Eiden and his coworkers have examined the regulation of neuropeptide (enkephalin and VIP) biosynthesis in cultured chromaffin cells. By using both RIA's and cDNA probes they have been able to study regulation of transcription, translation, and post-translational processing. Expression of both enkephalin and VIP seems to be mediated by changes in intracellular cyclic AMP.

Dr. Eiden has developed a radioimmunoassay for chromogranin A. He is in the process of preparing and screening a bovine adrenal medulla library for cDNA to chromogranin mRNA. He hopes to characterize this apparently ubiquitous secretory protein and to study its production in detail.

Dr. Pruss has prepared monoclonal antibodies to chromaffin granule membrane proteins and is using them to study membrane synthesis, compartmentation, and processing. In addition, Dr. Pruss has developed a method for preparing subcellular fractions of cultured chromaffin cells and is studying, with Drs. Hook, Eiden, and Hotchkiss, factors that influence differentiation of these cells.

Dr. Siegel is investigating the role that depolarization of secretory cells plays in controlling the synthesis of secreted products. She has prepared antibodies against the BAM-22 fragment of the enkephalin precursor.

Dr. Hirata and his colleagues have previously shown that arachidonic acid, a precursor of prostaglandins and leukotrienes, is released from membrane phospholipids after stimulation of various receptors. The arachidonic acid is derived from methylated phospholipids (phosphatidylcholine and/or phosphatidylinositol), suggesting that the activation of phospholipases has a key role in its production. The activities of phospholipases in a variety of cells were suggested to be controlled by phosphorylation-dephosphorylation of lipomodulin. Lipomodulin was isolated initially from rabbit neutrophils treated with glucocorticoids and recently from HL60, human promyeloleukemia cells. The isolated protein has glucocorticoid-like activities such as promotion of cellular differentiation, suppression of immunoglobulin synthesis and inhibition of chemotaxis. All these activities have been attributed to this protein's inhibition of phospholipases.

Annual Report of the Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

October 1, 1982 - September 30, 1983

Paul D. MacLean, M.D., Chief

In a seminal paper written early in his career, C. Judson Herrick emphasized the value of the comparative approach in investigating the anatomy and functions of the central nervous system. In particular, he noted, it has "the great power of the pathological method" because "phenomena are often simplified by being split up into their components." Indeed, he went on to say, "Pathological conditions are all experiments--nature's experiments--often more ingenious than we could invent." Over and over again, he pointed out, one sees in the nervous system of various animals "the analogies of pathological conditions with the only difference that they are spread out in time and extended along the phylogenetic pathway." Herrick was saying, in effect, that there is no experiment that nature has not done for us.

The comparative neurological approach, together with an examination of the fossil record, has revealed that the primate forebrain evolved and expanded to its great size while retaining the anatomical and biochemical features of three basic formations reflecting an ancestral relationship to reptiles, early mammals, and late mammals. The value of the comparative approach in experimental neuro-behavioral work is illustrated by investigations in our Laboratory subsequent to its occupancy in 1971. Hand in hand with our becoming aware that reptiles and mammals share the same four main kinds of behavior in social communication, we found that the corresponding parts of the brain (the striatal complex) in both classes of animals play an essential role in the integrated performance of communicative displays. Historically, the striatal complex of mammals has been regarded as the "head ganglion" of the motor apparatus under control of the neocortical motor areas, having, so to speak, "no mind of its own."

Subsequently, it became evident that three forms of behavior that distinguish the evolutionary transition from reptiles to mammals are represented in the cingulate gyrus that contains the evolutionarily most recent cortex of the limbic system. Inclusive of the cingulate gyrus, the limbic system represents an inheritance from early mammals. Significantly, there is no counterpart of the thalamocingulate structures in the reptilian brain (Le Gros Clark and Meyer, 1950). The behavioral triad in question comprises (1) nursing in conjunction with maternal care, (2) audiovocal communication for maintaining maternal-offspring contact, and (3) playful behavior.

From the standpoint of understanding human evolution, there could hardly be anything more important than reconstructing the steps by which the just mentioned forms of behavior developed. In taking a comparative approach to investigating the vocal aspects, we are utilizing squirrel monkeys for elucidating the neural substrate underlying the patterning and production of the isolation call. Such calls, which appear to be universal among mammals, probably represent the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. Occurring in both infant and adult squirrel monkeys, the isolation call becomes almost as stable as a

fingerprint by one year of age. The isolation calls of two main varieties of squirrel monkey have slight differences that are distinctly manifest in the sound spectrogram. Because of its unique features and its ready elicitation in adult monkeys when temporarily isolated, the call lends itself to neuro-behavioral studies. Our original findings revealed that core gray matter and adjacent tegmental structures at the junction of the thalamus and midbrain are essential for the patterning and production of the isolation call. Most notable in regard to the cingulate gyrus, it appears from this year's findings that a rostral strip of limbic cortex extending from the anterior cingulate to the base of the septum is essential for the spontaneous production of isolation calls. Other experiments indicate that mechanisms underlying the production of the call extend from the caudal thalamic core gray matter and adjacent tegmental fiber systems down to the caudal level of the inferior colliculus.

In view of the distressful nature of the isolation call, it is of special interest that opiate receptors are found in high concentration in both the core gray matter in question and in the cortex of the cingulate gyrus, and that morphine eliminates the call, while the morphine antagonist, naloxone, reinstates it. Relevant to this and the other mentioned cingulate functions, it has been reported that morphine disrupts maternal behavior. Such considerations suggest how the research under discussion may apply to problems of concern to mental health, as for example, separation anxiety, "failure-to-thrive," excessive grief reactions, depression, and opiate addiction. Apropos of the comparative approach to such matters, it is gratifying that the National Institute of Child Health and Human Development is enlarging its facilities next to our Laboratory, and with the cooperation of Mental Health, will be supporting an enlarged primate research group under the direction of Dr. Stephen J. Suomi, whose behavioral research on rhesus monkeys has included observations on separation reactions.

It has turned out that other projects of the Laboratory, including those of the Unit for Research on Behavioral Systems (URBS), have uncovered findings particularly relevant to maternal behavior and rearing of young. In earlier projects concerned with the neuroanatomy and neurochemistry of the striatal complex, it was found that the amount of pallidal iron in female rats is significantly higher than that in males, and that there are wide fluctuations of iron levels in both the pallidum and in the substantia nigra during the estrous cycle and in pregnancy. It is possible that the normally high content of iron in these parts of the brain helps to ensure a reserve in case of a prolonged nutritional or other deficiency of iron. It is known that iron deficiency, which ranks as the most prevalent nutritional disorder, results in the disturbance of various procreation-related functions, as well as psycho-behavioral alterations. New electron microscopic findings, described in this year's report, have provided additional evidence that the high iron concentrating areas of the brain overlap with neural systems known to be involved with the release of gonadotropic hormones. Evidence has also been found that interference with neurotransmitter metabolism of the corpus striatum results in a decrease of iron in the pallidum and substantia nigra.

In URBS, behavioral observations were completed this year on the effects of micrencephaly in rats living in two complex environments containing, respectively, four and eight adult pairs. Micrencephaly was produced by treating the mothers of the subjects with the alkylating agent methylazoxymethanol acetate on the 15th day of gestation. Such treatment results in a marked reduction in the development of the neocortex, as well as of the cingulate gyrus, particularly

in the parasplenic region. The experimental animals living in the environment with four pairs were, after a long period of adjustment, about 50 percent as successful as the control group in rearing pups to the age of weaning. In environments with eight pairs both the experimental and control animals had less reproductive success than these in the uncrowded environments, with the micrencephalic animals rearing only 10 percent of the pups from their first litters.

In concluding this summary giving special emphasis to comparative findings relevant to maternal-offspring behavior, mention will be made of some observations from one of two long-term investigations in URBS now involving an extensive computer-assisted analysis of the data. The purpose of one of these investigations was to learn whether or not rats living in a cooperative society (as illustrated by the need to cooperate in pairs for obtaining water) would fare better under conditions of crowding than control animals that were required only to look after themselves. One result was that under the conditions of cooperation, the females reared less than half the expected number of young. Somewhat like the working mother in a human society, the time required of a female with pups to engage in cooperative drinking and other activities kept her from the nest. As a consequence it appears that litters of pups were partially or completely destroyed. Nevertheless, the young that were reared were found to be heavier and in better condition than those nurtured by the controls. For purposes of survival, it seems, nature is prepared either to produce large numbers of animals or to strive for quality in a few.

Annual Report of the Laboratory of Cerebral Metabolism
National Institute of Mental Health

Louis Sokoloff, M.D., Chief

October 1, 1982 through September 30, 1983

The Laboratory of Cerebral Metabolism consists of two Sections with independent research programs in the area of biochemistry of the nervous system. Although their programs are quite distinct in their orientation, the two Sections share equipment, facilities, and methodology, particularly with respect to analytical biochemical procedures. The Laboratory suffers severely from inadequate space. Space restrictions result in allocations of facilities usually on the basis of expediency rather than rational organization of resources. The recent acquisition of several office modules has partially alleviated the pressure on the secretarial staff. The Laboratory, nevertheless, maintains an active research program which has achieved and retains a position of worldwide respect.

Section on Developmental Neurochemistry

Louis Sokoloff, M.D., Chief

This Section continues its research along three lines: 1) the refinement and extension of the deoxyglucose method for the measurement of local cerebral glucose utilization and its application to various physiological, pharmacological, and pathological conditions; 2) intermediary energy metabolism of the central nervous system; and 3) the development and applications of a method to measure local rates of protein synthesis in the central nervous system.

Experience with the deoxyglucose method has led to the recognition that it breaks down under conditions of severe hypoglycemia or hyperglycemia, far beyond the normal range of blood glucose concentration. Because a number of physiological, pharmacological, and pathological conditions are characterized by marked changes in the blood glucose level, it became important to analyze and adjust the method for this phenomenon. The problem has been localized to changes in the "lumped constant" and rate constants, components of the operational equation of the method. The "lumped constant" has been redetermined over the entire range of arterial plasma glucose concentrations from 40-600 mg% in studies carried out one to two years ago. Drs. F. Orzi and D. Dow-Edwards, at first, and most recently Drs. H. Namba, G. Lucignani, and A. Nehlig have now completed measurements of the rate constants and are using these constants to study the effects of plasma glucose concentration on local cerebral glucose utilization.

Previous studies with the deoxyglucose method on the effects of dopamine agonists demonstrated metabolic activation in all components of the dopaminergic extrapyramidal motor system. Surprisingly, no effects were seen in the mesolimbic dopaminergic system. These studies were carried out acutely with single doses of d-amphetamine or apomorphine. Drs. Orzi and Dow-Edwards examined the effects of chronic d-amphetamine administration by osmotic pumps. One week of administration results in increases of glucose utilization in the nucleus accumbens, in contrast to the lack of effect of a single dose. These results are consistent with the hypothesized basis of amphetamine psychosis, which is considered by some to be a model of schizophrenia. These studies were recently published.

The applications of the deoxyglucose method have been extended into the field of neuroendocrinology. Dr. Dow-Edwards has completed studies on the effects of cretinism on local cerebral glucose utilization. In rats radiothyroidectomized at birth but allowed to reach adult age, there are marked and widespread decreases in glucose utilization throughout the brain, but the cerebral cortex, particularly the primary sensory cortical regions, e.g., visual, auditory, and somatosensory, are by far the most affected. The results are in keeping with neuroanatomical studies which indicate that the neuropil of the cerebral cortex is underdeveloped in cretinism. Drs. Linda Porrino and Hiroki Namba are carrying out studies designed to relate local changes in cerebral glucose utilization to sexual behavior in the female rats. Local cerebral glucose utilization varies with the stages of the estrous cycle. Physiological doses of estradiol administered by chronic implants stimulate glucose utilization in a number of hypothalamic nuclei. Progesterone reverses this effect of estradiol while having relatively little effect of its own. The increases in glucose utilization in the hypothalamic nuclei caused by estradiol coincide with an increase in sexually receptive behavior.

Drs. Massako Kadekaro and Paul Gross, in collaboration with Dr. Juan Saavedra, are studying local cerebral glucose utilization in a genetic strain of rats with a defect in vasopressin synthesis (Brattleboro DI rat). They have found that despite the deficiency in vasopressin synthesis, the hypothalamico-hypophyseal tract, especially the posterior pituitary and subfornical organ, is metabolically activated. Replacement treatment with vasopressin, which reverses the symptoms of diabetes insipidus exhibited by these animals, does not reverse the metabolic activation. The mechanisms underlying the metabolic activation are under study. Angiotensin II is known to be elevated in the plasma of Brattleboro rats, and it is known to act on the subfornical organ to stimulate drinking behavior. Recently completed studies have shown that infusions of angiotensin II do, in fact, stimulate glucose utilization in the subfornical organ and the posterior pituitary. These results are currently being prepared for publication.

In the course of earlier studies on the effects of electrical stimulation of afferent inputs on glucose utilization in the superior cervical ganglion, Dr. M. Kadekaro noted that urethane anesthesia itself stimulated metabolic rate in the ganglion but not in brain where it, like other anesthetics, depressed glucose utilization. Dr. Masanori Ito and Dr. Kadekaro pursued this observation and found that the urethane effect on the superior cervical ganglion is dependent on humoral factors; adrenalectomy blocks the effects on the ganglia but not the brain. Catecholamine levels in plasma are normally found to be increased in urethane anesthesia. The scenario appears to be as follows. Urethane anesthesia, commonly used in neurophysiological studies, stimulates sympathetic activity and release of catecholamine hormones from the adrenal medulla. These hormones enter the superior cervical ganglion, which has no blood-tissue barrier, and activate energy metabolism. The hormones do not, however, affect metabolism in the brain because they are excluded from the tissue by the blood-brain barrier.

Dr. Carolyn B. Smith has completed her studies on the influence of aging in the central nervous system of rats. In previous work she found age-dependent selective reductions in glucose utilization in specific regions of the brain. Particularly affected were the components of the primary visual and auditory components, similar to effects seen following blockade of the primary sense organs. These results suggested that some of the central nervous changes in aging might be secondary to sensory deprivation or deafferentation. Also affected by aging were

components of the dopaminergic nigrostriatal pathway. These are structures in which glucose utilization can be activated by dopamine-agonists, such as d-amphetamine and apomorphine. In recently completed studies Dr. Smith has found decreased responsivity of these dopaminergic structures to apomorphine with age. These results suggest a loss of functional dopamine receptors in the nigrostriatal system with aging and may offer a basis for the development of senile parkinsonism. These studies are currently being prepared for publication.

A major research effort of the Section is directed at the development of a method for the measurement of local rates of protein synthesis in the nervous system. This technique is designed to be an autoradiographic procedure and utilizes some of the principles developed for the deoxyglucose method, but it is far more complex because of the special biochemical properties of the protein-synthesizing system. The development of the method and some of its applications are under the direction of Dr. Carolyn B. Smith. The problems are the possibility of intracellular compartmentation of the amino acids used for protein synthesis and the recycling of amino acid derived from protein breakdown, and she has designed progressively more complete and complex kinetic models to take these phenomena into account. She has also derived the appropriate operational equation to go with each model. The completion of the development of the method awaits only the clarification of which of these models is most appropriate. Last year, an experimental procedure was designed that will provide this clarification. The procedure consists of a programmed infusion of [^3H]amino acid to provide a constant arterial concentration for a variety of intervals, a different animal for each interval. At the end of the interval the animal is killed, the brain removed, and the tRNA-amino acids extracted from the brain tissue. The tRNA-amino acid must be purified by a variety of steps, and then deacylated, and the free [^3H]amino acids are then dansylated with [^{14}C]dansylchloride. The specific doubly labeled dansylated amino acid is then separated by high pressure liquid chromatography, and the $^3\text{H}/^{14}\text{C}$ ratio measured in a liquid scintillation counter. The amino acid in the plasma is similarly treated to determine the $^3\text{H}/^{14}\text{C}$ ratio. This procedure will provide the rate constants for the equilibration of the precursor pool amino acid with that of the plasma and from the equilibrium $^3\text{H}/^{14}\text{C}$ values of the precursor amino acid compared to that of the plasma amino acid will also provide the evidence for the presence or absence and the degree of recycling of amino acid from protein breakdown. This procedure consists of a number of complex chemical procedures, and Dr. Smith has systematically worked out all the details of each step. These and alternative studies to solve the above question are now in progress. The results thus far indicate very rapid turnover of the precursor amino acid pool for protein synthesis and its rapid equilibration with the amino acid pool in the plasma. Studies are continuing on the question of the degree of amino acid recycling from protein breakdown.

This final series of experiments on the modelling is required only to achieve absolute quantification of the rates of protein synthesis. Even without them, the first model designed by Dr. Smith can provide a reasonable estimate of the rates of protein-synthesis which are adequate to examine some biological processes. For example, Dr. Smith has used the method to study regeneration in the hypoglossal neural pathway. Section of the hypoglossal nerve in the rat is followed within 4 days by increased protein synthesis in the hypoglossal nucleus which persists until the nerve has regenerated fully and has become functional. The increased protein synthesis is preceded by an increased rate of glucose utilization in the nucleus. The mechanism of the increased energy metabolism and its relationship to

the increased protein synthesis and process of regeneration is under study. Last year the method was used to study plasticity in the visual system of the newborn monkey. Acute patching of one eye had no effects on protein synthesis anywhere in the brain, but a chronic patch of approximately 3 weeks resulted in a decreased rate of protein synthesis in the lateral geniculate laminae normally served by the deprived eye. Inasmuch as the protein synthesis required for axonal sprouting and growth of the geniculostriate pathway resides in the cells of the lateral geniculate, this result provides evidence that the loss of ocular dominance columns in the visual cortex following unilateral eye patching is the result of reduced protein synthesis and axonal growth in the pathway for the deprived eye. Dr. Smith is continuing studies of this phenomenon in monkeys to determine the age range of plasticity, the effects of reverse patching, etc. The method is also being used in studies of slow wave sleep in the adult monkey to determine whether there are changes in protein synthesis in any specific structures. The results thus far indicate decreased protein synthesis throughout the brain, except for 3 regions with increased protein synthesis in sleep -- i.e., locus coeruleus, area postrema, and substantia innominata.

Drs. Elaine Kaufman and Thomas Nelson are continuing their biochemical research on the origins, disposition, and functional significance of γ -hydroxybutyrate in brain. This compound is normally present in brain, but when administered in pharmacological doses, it produces a trance-like state and a marked generalized depression of cerebral energy metabolism. Reports in the literature suggested that the behavioral effects of the compound are blocked or diminished by naloxone; Drs. Kaufman and Nelson in collaboration with Dr. Gregory Crosby, a previous Guest Worker in the Laboratory, have found that naloxone also antagonized its metabolic effects. These results suggest that opiate receptors may be in some way involved in the mechanism of its behavioral and metabolic effects. Drs. Kaufman and Nelson have also made significant progress in elucidating the biochemical origin of the compound. γ -Hydroxybutyrate had been believed to be derived from the metabolism of γ -aminobutyric acid, but Nelson and Kaufman have found that γ -hydroxybutyrate is present in even larger amounts in extraneural tissues, like kidney or liver, which lack glutamic decarboxylase, the enzyme responsible for the formation of GABA. In recent studies with labeled substrates they have obtained evidence that γ -hydroxybutyrate is derived from GABA in kidney also, but the GABA is generated from polyamines, such as putrescine, and/or arginine. A series of enzyme reactions involving pyridine nucleotides are responsible for this alternative pathway for GABA formation. Studies are continuing on the properties of the enzymes involved in γ -hydroxybutyrate synthesis and oxidation. Recent studies with inhibitors of γ -hydroxybutyrate dehydrogenase, an enzyme first isolated and purified by Kaufman and Nelson, indicate that these inhibitors can enhance the tissue levels of γ -hydroxybutyrate. Inasmuch as some of these inhibitors are metabolically formed in the course of the oxidation of naturally occurring amino acids, it appears that the level of γ -hydroxybutyrate in the tissues can be regulated by normally occurring metabolic processes.

The deoxyglucose method depends on the trapping of the label in deoxyglucose in deoxyglucose-6-phosphate or any other compound derived from it. There have been recent reports in the literature that deoxyglucose is also incorporated into glycogen. Dr. Thomas Nelson has systematically examined this reaction and has found that deoxyglucose is indeed incorporated into glycogen but relatively slightly. In the course of a typical deoxyglucose experiment less than 1-2% of

the label is in glycogen; the remainder is in deoxyglucose-6-phosphate. With the usual quantitative autoradiographic procedure employed, this incorporation into glycogen is of no significance to the results. There have, however, been attempts to increase the resolution of the method by fixation of the tissue. Fixation results in major loss of the water-soluble deoxyglucose-6-phosphate while the labeled glycogen is retained. Under these circumstances the deoxyglucose method does not measure glucose utilization, but is only a stain for the presence of glycogen. These studies have been completed, and manuscripts are being prepared for publication.

Section on Myelin Chemistry
Marian W. Kies, Ph.D., Chief

The discovery of myelin basic protein and its subsequent purification by the Section on Myelin Chemistry has developed into a major program involving both neurochemical and immunologic studies on myelin basic protein (BP). Having isolated the "antigen" responsible for experimental allergic encephalomyelitis (EAE), we asked the questions "How is the disease induced"? and "Why is the basic protein so antigenic"?

This led us to study many aspects of the pathogenesis of EAE. The delayed skin reaction to BP (comparable to the tuberculin test for tuberculosis) was shown to be correlated with disease severity and supported the theory that EAE was caused by cellular sensitization. We also showed that BP in incomplete Freund's adjuvant prevented and/or suppressed the disease in an immunologically specific manner. By direct isolation of BP from purified myelin we demonstrated its in vivo localization.

Recently we have completed some of our most important studies on the pathogenesis of EAE. About 4 years ago, Dr. Driscoll showed that BP-sensitized cells (either lymph node or peritoneal exudate) cultured with BP in vitro were markedly enhanced in their ability to transfer EAE.

As a result of this technique (enhanced cell transfer), we have been able to induce chronic EAE by a combination of passive transfer and active sensitization in a manner more suitable for studying demyelination than techniques previously employed. We have also demonstrated strain specificity for encephalitogenic site recognition in inbred guinea pigs -- Str 2 guinea pigs are sensitized by a site in the N-terminal half of the BP molecule, whereas Str 13 guinea pigs are sensitized by the tryptophan site (Res 113-122) in the C-terminal half of the molecule. This was the first example of strain specificity with respect to the encephalitogenic site. In addition, with this technique Dr. Driscoll has demonstrated that cells participating in the in vitro enhancement and transfer of EAE are Ia⁺ (that is, they express the Ia antigen on their membranes). He has shown that soluble factors are required for the transfer of EAE and that more than one subset of cells is required to achieve enhanced transfer. The latter observations are of significance not only for EAE research but also represent important advances in cellular immunology. In this research, Dr. Driscoll and Dr. Kies have had the skillful assistance of Ms. Judy Parkison and Ms. Martha Bacon. Several post-doctoral fellows have also participated in these cell studies -- in the past, Dr. John Richert and Dr. Tadashi Namikawa; currently, Dr. Takashi Fujii.

Parallel to the cell studies, Dr. Martenson's and Ms. Deibler's research on BP has contributed important basic information on its chemistry and structure. Their observations have often been essential for the successful completion of an immunologic experiment (e.g., the elucidation of strain-specific encephalitogenic sites).

During the past year Dr. Martenson has pursued several avenues of work. His major effort has been to investigate specific enzymatic cleavages of BP to obtain suitable fragments for biochemical studies. Because of his knowledge of BP and its structure, Dr. Martenson has been very skillful at producing fragments "made to order" for a specific job. He has prepared and identified peptides from thrombin and/or pepsin digests of BP using either limited digestion for large fragments or more extensive digestion for smaller fragments. These have been used for (1) immunologic studies to define strain specificity (as described above); (2) other immunologic studies to define the binding-specificity of monoclonal antibodies; (3) physicochemical studies to elucidate the detailed conformation of different regions of the molecule; and (4) biochemical studies on the catalytic activity of the protein (intrinsic esterase activity). In addition, he has embarked on a study of the amino acid sequence of porcine BP (used in one of the recent "treatment trials" on MS patients). Dr. Martenson has been assisted in this research by Ms. Mona Pedersen, Ms. Gladys Deibler, and Dr. Junichi Kira.

In addition to her participation in the studies summarized above, Ms. Deibler has re-examined the large-scale procedure for isolation of BP in semi-purified form. Most basic proteins (bovine, guinea pig, rabbit and monkey) are easily purified from this batch preparation. Human BP, on the other hand, has presented a unique problem because of its contamination with a neutral proteinase of unusual stability. Recently we found that rat BP also is contaminated with a proteinase. In attempts to remove the enzyme, the batch procedure has been modified so that component 1 of human BP can be isolated and purified. In a careful examination of purified vs. crude BP, Ms. Deibler demonstrated that the proteinase was removed by the new procedure but that all purified BPs are susceptible to a certain amount of hydrolytic cleavage (non-enzymatic) which is enhanced by raising the pH to 9 and the temperature of incubation to $\sim 100^{\circ}\text{C}$. She has been assisted in this study by Ms. Lisa Boyd.

The combined efforts of the scientists of the Section, and their assistants, have contributed significantly to our understanding of myelin BP and the role it plays in both normal and abnormal reactions of the nervous system.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health
October 1, 1982 to September 30, 1983
Giulio L. Cantoni, M.D., Chief

Fiscal year 1983 has been relatively uneventful from an administrative point of view. However, continued budgetary limitations have imposed serious obstacles to the optimal development of our research capabilities. We have been able to meet most of our needs from other object funds, by careful monitoring of our expenditures and postponement of some major purchases; however, the scope of our research efforts has been seriously and adversely affected by administrative actions that have prevented us from recruiting professional staff to replace younger collaborators lost through normal attrition. In FY'83 the size of the professional staff supported by NIMH has shrunk by 25% (from 12 to 9); while we have been able to benefit from recruitment of guest workers supported by outside funds, it is clear that the reduction in the level of support by NIMH will, in the long run, have an adverse effect on the scope and strength of our research program. In spite of these limitations it is gratifying to report that good progress has been made in many areas of research.

Dr. Cantoni and his collaborators (Drs. Aksamit, Backlund, Bozzi, and De la Haba, at NIMH; Drs. Schecter and Dean, at NHLI; Drs. Scarpa and Strom, at the University of Rome, Rome, Italy; and indirectly, Drs. Stramentinoli and Camozzi, at Bio Research, Milan, Italy) have continued to investigate the biochemistry and biology of S-adenosylmethionine.

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under an intensive study in this and other laboratories. Studies on purified AdoHcyase from several sources have shown that this is a complex enzyme, made up of four subunits, which bind NADH, adenosine and cAMP.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccha-

rides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. It can be surmised that modulation of AdoMet/AdoHcy ratio would result in important physiological effects, which if correlated with biochemical data would help reveal the significance of specific methylation reactions.

In prokaryotes, the isolation of mutants has helped to analyze the functions of specific biochemical reactions. In eukaryotes, isolation of mutants is more difficult, so other approaches have been devised, such as using inhibitors to block specific reactions. Since AdoHcyase is the only enzyme to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula $\text{S-purinyldihomocysteine}$ (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have shown that inhibitors of AdoHcyase may be divided into two groups: a) irreversible or suicidal inhibitors, and b) competitive inhibitors that inhibit the enzyme reversibly. This second group can be further classified into two subgroups; those inhibitors which can be utilized as substrates by the enzyme and those inhibitors which are not substrates. Several compounds have been examined which are irreversible inhibitors of AdoHcyase, and include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deaza-aristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μM , and as a substrate it is about equivalent to the natural substrate, adenosine with a similar affinity for the enzyme. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

In vivo, administration of 3-deaza-Ado to laboratory animals or cells in culture results in the accumulation of both 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) and AdoHcy. The accumulation of 3-deaza-AdoHcy can be increased by addition of homocysteine, due to the fact that AdoHcyase is present in cells in a very large excess over the rate limiting concentrations. The enzymatic equilibrium favors synthesis and 3-deazaadenosine, an adenosine analog that is resistant to the action of adenosine deaminase is rapidly and quantitatively converted to 3-deazaadenosine. By contraction, the accumulation of AdoHcy brought about by administration of 3-deaza-Ari, and other potent inhibitors of AdoHcy reflects the rate of transmethylation reactions and is not influenced by addition of homocysteine. Differences between species were observed for the metabolite levels formed after treatment with these compounds. Administration of 3-deazaadenosine to rats pro-

duced accumulation of both 3-deaza-AdoHcy and AdoHcy, however, the same treatment in hamsters resulted only in an accumulation of 3-deaza-AdoHcy. Examination of the kinetic properties of the enzyme from rat and hamster liver revealed that the K_m for AdoHcy is ten times smaller for the hamster enzyme than for the rat. This could explain the lack of AdoHcy accumulation in hamster liver.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-deaza-AdoHcy, with the accompanying changes in the AdoMet/AdoHcy ratio, would result in the inhibition of a number of transmethyases. This should cause an increase in the intracellular level of AdoMet (as a consequence of its under-utilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify this prediction, demonstrating a striking decrease in the amount of many methylated compounds, including methylated phospholipids, methylated proteins, and creatine in the liver.

Studies in this and other laboratories on a large number of analogs of AdoHcy analogs have demonstrated a wide range in the sensitivity of different transmethyases to inhibition by these compounds in vitro. Unfortunately, cellular membranes are relatively impermeable to AdoHcy and its analogs, so it has been difficult to take advantage of the specificities of these analogs in vivo. However, the capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly.

Since 3-deazaaristeromycin has such a low K_i for AdoHcyase, high concentrations of this compound should effectively block the enzyme, preventing the conversion of AdoHcy to adenosine and homocysteine. Comparison of the effects of 3-deaza-Ado and 3-deaza-Ari on the replication of RAW264 cells showed that, at sufficiently high concentrations, 3-deaza-Ado was cytolytic after one day and that 3-deaza-Ari was cytostatic. Micromolar amount of homocysteine reversed the cytostasis of 3-deaza-Ari, but did not reverse the cytotoxicity of 3-deaza-Ado. We concluded that the cytotoxicity of 3-deaza-Ado is likely mediated by 3-deaza-AdoHcy, while on the other hand, cytostasis of 3-deaza-Ari was due to a profound inhibition of AdoHcyase. Since AdoHcy is the only cellular source of homocysteine, cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in de novo synthesis of purines and pyrimides. This condition is similar to the situation with vitamin B₁₂ deficiency, which inactivates methionine synthase, and causes methyltetrahydrofolate to accumulate. In addition, it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari bring about a significant inhibition of methylation of phosphatidylethanolamine in these cells, which rules out a requirement for this reaction in chemotaxis.

In the same manner, both compounds inhibit protein lysine and arginine methylation and carboxylmethylation to the same extent. We have further shown that inhibition of chemotaxis by 3-deaza-Ado is correlated with changes in incorporation of radioactive assays and the synthesis of specific proteins which are not affected by 3-deaza-Ado.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibit the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated RNA than 3-deazaaristeromycin.

One of our goals is to determine which biochemical reaction(s) in chemotaxis is inhibited by treatment of the cells with 3-deazaadenosine. As a first step toward this goal, we observed the motility of RAW264 cells by time-lapse videocinematography. RAW264 cells were motile and morphological changes occurred when the cells were exposed to attractant. Incubation of cells with 3-deazaadenosine, under conditions that inhibited chemotaxis by approximately 90%, did not noticeably affect motility or the response to attractant. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after binding to the chemoreceptor is inhibited.

Both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines, suggesting that a methylation reaction may be involved in altering gene expression in differentiation. Again, there are differences between these two compounds in their ability to induce differentiation in different systems. In collaboration with Drs. Scarpa, Strom, and Bozzi, it was found that 3-Deaza-Ado will increase the rate of myoblast fusion to form myotubes when the cells are placed in a permissive fusing medium. In addition, non-fusing variants of myoblast cells, which normally do not fuse in a permissive fusing medium, will fuse when 3-deaza-Ado is added. Work from several other laboratories has suggested that DNA methylation may be involved in expression of specific genes. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. However, since 3-deaza-Ado also inhibits a number of other methylation reactions, further work will be required to identify the mechanism for this effect of 3-deaza-Ado.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, amitriptyline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use.

The mechanism of action of AdoMet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

A project designed to study some of the possible metabolic consequences of AdoMet administration to human volunteers is in its very early stages in collaboration with Drs. W. Potter and M. Sherer.

During the past year, work in the Section on Alkaloid Biosynthesis has continued to focus chiefly upon the aspartate biosynthetic pathway in higher plants. This pathway is the chief means of production in nature of four amino acids essential for man and other non-ruminant animals: methionine, lysine, threonine and isoleucine. The first three of these amino acids may limit the nutritive value of diets deficient in animal proteins, such as are eaten by millions, perhaps billions, of individuals in the world today. Essential amino acid deprivation during infancy has been implicated as a major cause of irreversible mental retardation. In-so-far as our work provides insight into how these amino acids are made, and how their production is regulated and interrelated to other physiological events, rational optimization of such production may become possible.

Here we will mention only the highlights of our recent work and indicate very briefly possible directions for future work. Drs. Giovanelli and Thompson have provided semipurified preparations of threonine synthase and cystathionine γ -synthase, the enzymes catalyzing the crucial committing reactions competing for O-phosphohomoserine and leading ultimately to threonine and isoleucine on the one hand, or to methionine on the other. The two enzymes are physically separable. Their substrate specificities have been studied. The properties of cystathionine γ -synthase enable us to understand the role of O-phosphohomoserine sulphydrylase and of the O-acylhomoserine utilizing activities previously detected in crude plant extracts, and until now of puzzling physiological significance. Further purification of cystathionine γ -synthase would be most important in permitting studies of the molecular mechanism of its regulation. Raising antibody to this enzyme would permit studies of its *in vivo* synthesis and processing, and cloning of c-DNA or the gene locus for this enzyme.

Threonine synthase is allosterically activated by AdoMet. Studies of the mechanism of this activation are planned. Threonine synthase is specifically and markedly inhibited by 5'-AMP and inorganic phosphate at physiological concentrations. A new and significant area for regulatory effects is thereby revealed.

Dr. Datko's studies of sulfate uptake have now shown that the physiologically important system (the "low K_m " form) is probably controlled by both sulfate and cysteine. To fully understand how this complex regulation works, and many other features of sulfur metabolism by plants, attempts will have to be made to deal experimentally with possible subcellular compartmentation of both sulfate and cysteine, indicated by labeling studies. Dr. Datko's studies of uptake of organic compounds have proven the presence of a multitude of channels of surprising specificity. The purine channel, the choline channel, the ethanolamine channel, and the urea channel had not been previously recognized in plants. Their exquisite specificity strongly suggests they are physiologically meaningful to the plant, and reassure us that administration of compounds via these channels is not an entirely artificial procedure. The understanding provided by these studies will be invaluable in rational design of a variety of experiments in which these compounds are fed, singly or in combination, to Lemna.

Work on the international survey of patients with homocystinuria due to cystathionine β -synthase deficiency has progressed satisfactorily. An unexpectedly large data-base has been accumulated. Most of the problems inherent in analyses

of these extensive, and necessarily unsystematic, data have now been dealt with. Although final results are not yet available, it is certain that the study will accomplish its chief aims: to provide base-line data for untreated patients with respect to mental retardation, thromboembolic episodes, lens dislocation, survival, and appearance of osteoporosis. These data will provide the necessary means to evaluate therapies as data accumulates in the future. Already, encouraging indications are available that methionine restriction, initiated sufficiently early, has prevented mental retardation in a group of patients who otherwise would all surely have been retarded; and that B₆ treatment of responsive patients is strongly indicated to effectively prevent the majority of thromboembolic episodes which would otherwise have occurred. These and a variety of other results which should be forthcoming, should be of lasting value to patients with cystathionine β -synthase deficiency.

Klee and his colleagues have continued their studies aimed at clarifying the structure and function of opiate and related receptors. Purification of receptor components, a major goal of the group, has received an important boost from the synthesis by Rice, Jacobson and their colleagues (LC, NIAMDD) of a number of irreversible opiate ligands which form covalent bonds with recognition units of the receptors. Several of these are highly selective towards different receptor subtypes. Radiolabelled fentanylisothiocyanate was found to specifically react with a 58,000 M_r glycoprotein subunit of the opiate receptor (δ) present in neuroblastoma x glioma hybrid cell membranes. This protein has been partially purified, and after full purification, is expected to serve to provide amino acid sequence information and as an antigen with which to prepare anti-receptor antibodies.

As a result of their work on function of receptors, it has become clear that opiate receptor action involves, as a minimum, a receptor, or recognition protein, and an effector protein which is the GTPase turn-off protein of the adenylate cyclase complex. The recognition protein and GTPase are separated in detergent (CHAPS) extracts of membranes even though receptors in such extracts are still sensitive to GTP modulation. Thus, a third component of the system is a GTP binding protein which may couple opiate binding to GTPase stimulation. It has now become clear that the coupling protein for opiate receptors, N_i, is also used by other inhibitory receptors (α_2 adrenergic, muscarinic and somatostatin) and is different from that (N_s) which couples stimulatory receptors to adenylate cyclase. Experiments with bacterial toxins which modify one or the other of these regulatory proteins have led to the conclusion that N_i and N_s do not act completely independently but are functionally coupled. Binding of opiates to receptors was found to be regulated by N_i, as is GTPase stimulation, and the accompanying inhibition of adenylate cyclase. Each of these activities has been used to assay N_i reconstitution into pertussis toxin inactivated membranes as a necessary tool for purification of the protein. Reconstitution of all receptor functions after solubilization, a long term goal of the project, has received a considerable boost by the development of methods for the incorporation of GTP sensitive opiate binding activity into phospholipid vesicles.

Dr. Merrill and collaborators have continued to explore the applications of new biochemical methodologies to human genetic diseases affecting the central nervous system.

A concentrated effort has been made with the techniques of high resolution two-dimensional electrophoresis (2DE) autoradiography and ultra sensitive silver

staining. Initial studies involved a disorder with a known biochemical basis, the Lesch-Nyhan syndrome. Currently, we are investigating a disease with a known genetic basis, but no known trait specific markers, Huntington's disease, and a disease with suspected genetic basis, familial Alzheimer's disease. Large multi-generational pedigrees have been utilized. An Alzheimer family of 288 individuals (with 55 cases of dementia) is being studied. We have established skin fibroblast cultures, for biochemical and genetic studies from 68 individuals in this family. The Huntington family being surveyed is located at Lake Maracibo, in Venezuela. This family contains a very large number of individuals (approximately 1,500). This family is being studied in collaboration with Dr. James Gusella at MIT. We have established lymphoblast cell lines from 51 individuals in this family.

These pedigrees serve a dual role, they permit a greater chance to establish trait specific markers than smaller families would, and they also serve as a resource in the development of a human genome map based on polymorphic markers. These 2DE studies have identified 35 new polymorphic markers from a preliminary survey of 354 proteins in these pedigrees. The establishment of a human genome map based on polymorphic marker loci will be an invaluable aid in investigations of any disease which contains a genetic component. In an initial survey of 354 or 18% of the proteins visible on the 2DE gels from these pedigrees, we have identified 35 polymorphisms. With enhanced methods to analyze all of the visible proteins (approximately 2,000), we estimate that 360 polymorphic proteins should be present. This number of polymorphic markers would greatly facilitate the construction of a human genome map. Previous calculations demonstrated that 200 polymorphic markers would permit a human genome map, in which 80% of the genome is covered by polymorphic markers at 10 centimorgan (cm) intervals, a polymorphic marker within 10 cm of a disease related gene would have a 90% chance of being linked to the disease related gene. The protein polymorphisms identified in this study will complement the DNA polymorphisms which are being identified in other laboratories. These cell lines which were established from these pedigrees are permitting investigations of additional paradigms for these diseases, such as the possible role for viral genes, which have been integrated into the human genome. Large numbers of cells have been grown from each of 58 individuals in the Alzheimer family for DNA hybridization studies. These DNA's will be screened with biotin-labelled viral probes to detect the presence of viral related nucleotide sequences in the genomic DNA of man. The possibility of an association between viral integration into the chromosomes and the occurrence of inherited diseases, such as Alzheimer's disease, will be examined using these methodologies.

The Alzheimer pedigree will also be utilized along with tissues obtained from young and aged individuals to investigate the occurrence of amplification and excision events in the mitochondrial genome as a function of the aging process. These events in themselves may contribute to the symptoms of aging or it may be the synthesis of circularized plasmids from excised material which may play a role in the state of senescence. These plasmids are capable of existing in a free form in the cytoplasm and as integrated sequences in the nuclear chromosomal DNA. The presence of these plasmids will be examined using cloned mitochondrial probes and an attempt will be made to determine their relationship with aging and senility.

Dr. Cantoni's contributions to biochemical research was recognized by his election to the National Academy of Sciences this spring. He was also elected to the American Academy of Arts and Sciences in 1982.

Our senior scientists continue to participate actively, often at no expense to the

Government, in presenting lectures at various universities, and also in national and international meetings, including: American Society of Biological Chemists; NATO Advanced Study Institute Targets for the Design of Antiviral Agents Meeting, in Les Arcs, France; Tables Rondes Roussel Uclaf on Chemotaxis, in Paris, France; Gordon Research Conference; Neuropeptide Conference; International Conference on Cyclic Nucleotides and Protein Phosphorylation, in Milan, Italy; Society for Neurosciences; American Society of Plant Physiologists; and Sulfur Conference, in Bielefeld, West Germany.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
October 1, 1982 to September 30, 1983
Ichiji Tasaki, M.D., Ph.D., Chief

The goal of the research activities in the Laboratory of Neurobiology is to elucidate the nature of the excitation processes in nerve fibers and cells on a macromolecular basis. Both physicochemical and biochemical approaches are being employed to achieve this goal. Since these excitation processes form the basis for the physiological function of the nervous system in normal as well as abnormal conditions, studies of these processes are relevant to the program of the Institute. At present, the following three research projects are being carried out.

1. Detection and interpretation of mechanical changes in the nervous system associated with the process of excitation. (I. Tasaki, K. Iwasa, and P. Byrne)

Since the discovery of the phenomenon of swelling of invertebrate axons during excitation in this laboratory, we have established the following basic properties of the mechanical changes in the nerve fiber:

- (1) The peak of swelling (i.e., lateral expansion) coincides fairly accurately with the peak of the action potential recorded at the site of mechanical recording.
- (2) The swollen state of the axon travels longitudinally at the speed of the nerve impulse.
- (3) Conduction of a nerve impulse along an axon is associated with a decrease in the length of the axon. [This decrease is an expression of "swelling-retraction" well-known in colloid and polymer chemistry.]
- (4) Hyperpolarization of an invertebrate axon by application of an electric current produces shrinkage of the axon. Depolarization by means of electric current or by external application of potassium salt brings about swelling.

There is little doubt that these mechanical changes are directly related to the membrane conductance changes, heat production, intracellular potential changes, etc. and are fundamental to the understanding of the excitation processes on the molecular basis. During the year just past, we have made a substantial progress in analysis of the physicochemical basis of these mechanical phenomena.

During the past six months, we have expanded our research to include detection and analysis of mechanical changes in the vertebrate nervous system.

By using bullfrogs and toads, we found that the nerve cell-body in the spinal ganglion swells when excited by way of the dorsal root or the sciatic nerve. The magnitude of the observed pressure rise was comparable to the value encountered in invertebrate axons (namely, a few dyn/cm²). Undoubtedly, this mechanical change in the cell-body is analogous to the phenomenon of swelling in invertebrate nerve fibers.

We found also that the spinal cord swells upon arrival of a volley of afferent impulses. This swelling is accompanied by a simultaneous reduction in the length of the cord. These mechanical changes in the cord are so large that it could be detected by connecting the output of the mechanoelectric transducer directly to an oscilloscope. The time-course of the mechanical changes in the spinal cord was long, being of the order of 100 msec in duration. The effects of polarizing currents and of various neuropharmacological agents were studied by taking mechanical changes as an index.

From the results of these studies we are led to believe that the aforementioned basic properties of the mechanical changes in the nerve fiber applies to the changes observed in the vertebrate nervous system. We expect that further studies of these mechanical changes will provide us important information about the physiological events that take place in the spinal cord upon arrival of nerve impulses.

2. Regulation of protein and enzyme function by modulator sites on complex carbohydrates. (A. L. Stone)

Heparin and heparan sulfate are intracellular and cell surface glycosaminoglycans (mucopolysaccharides). Heparin is noted for its therapeutic use as an anticoagulant and is known to modify numerous other reactions involving enzymes. Heparin is able to exert its action either directly on an enzyme or indirectly, by binding to an inhibitor of an enzyme. Heparan sulfates are heparin-like molecules that are present in cell membranes (including CNS) and are implicated in cell-cell interactions, cell migration and fixation of enzymes to cell membranes. It is speculated that the multifunctional reactivity exhibited by these mucopolysaccharides is dependent upon the chemical properties of various unique oligosaccharides that have recently been shown to reside within the macromolecule. By virtue of their polyanionic nature, these mucopolysaccharides are also involved in binding and exchange of simple and complex cations in interstitial elements. We have used circular dichroism spectroscopy to investigate the molecular basis of modulation of the biological activity of several proteins by heparin and heparin-like molecules.

One type of modulation that we have studied is the acceleration of the reactions between antithrombin (protease inhibitor) and all the serine esterases of the clotting cascade. Heparin molecules have at least two functional domains that are able to modulate differentially the anticoagulant potency of antithrombin. The first domain contains a unique tetrasaccharide that is essential for the binding of heparin to antithrombin and that is capable of accelerating the inhibitory reaction between antithrombin and factor X_a . The second domain is found within ten saccharides from the first and is required for the acceleration of reactions between antithrombin and factors XI_a , IX_a , and thrombin. Our circular dichroism data has shown that these two modulators of heparin interact with different regions of antithrombin. The first modulator of heparin perturbs at least one of the exposed and one of the buried tryptophane residues of antithrombin while the second alters the interactions between a disulfide bridge of antithrombin and its neighboring amino acids. Thus, the multifunctional anticoagulant action of heparin is probably related to its ability to induce conformational changes in antithrombin.

We have also elucidated the saccharide sequence of the second functional domain of this heparin, using low ultra violet circular dichroism spectroscopy along with model compounds. This has enabled us to propose a tentative, complete sequence of the highly active, 6500 molecular weight fraction of heparin for the first time. The two functional domains were seen to be composed of structurally similar regions of relatively lower anionic density flanked by a different number of fully sulfated disaccharides. Thus, the multifunctional modulator consists of alternating regions of higher and lower anionic density and varies in the distance between the highly sulfated segments. This important structure-function relation may have general biological significance.

The metachromatic binding of cationic dyes to mucopolysaccharides induces extrinsic Cotton effects in the absorption bands of the bound dyes. The experimental circular dichroism spectra of these effects vary with the structure of the polymer. To further analyze the experimental spectra we have developed a computer program for calculating theoretical spectra predicted for the geometries of the various anionic groupings in the oligosaccharides.

Sanfilippo mucopolysaccharidoses are a series of genetic disorders which involve the accumulation of heparan sulfates, atrophy of central nervous system tissue and concomitant mental retardation. We have isolated oligosaccharides from patients with such diseases and plan to test these heparan sulfate fragments as putative modulators in a developing neurobiological system.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1982 THROUGH SEPTEMBER 30, 1983

Seymour Kaufman, Chief

During the last year, one of the major research efforts of the Laboratory of Neurochemistry has been the study of the mechanisms involved in the regulation of the activity and levels of the components of the aromatic amino acid hydroxylating systems, with special emphasis on the hepatic phenylalanine hydroxylating system. As has been discussed previously, one of the reasons for this emphasis is that it is known that a normal functioning phenylalanine hydroxylating system is essential for normal brain development.

We have expanded our understanding of the mechanisms by which phenylalanine can regulate the activity of this enzyme. It has been established by us, as well as by others, that phenylalanine can directly activate phenylalanine hydroxylase. In addition, phenylalanine, in all probability, is also involved in the glucagon-mediated activation of the enzyme. We have previously shown that this activation involves the glucagon stimulation of hepatic adenylate cyclase, which, in turn, leads to the activation of cAMP-dependent protein kinase. Phenylalanine is also implicated in this regulatory mechanism because of its known ability to stimulate the release of glucagon from the pancreas.

We have now shown that phenylalanine plays another key role in this cAMP-dependent protein kinase-mediated activation of the hydroxylase because of its ability to overcome the inhibition by tetrahydrobiopterin (BH_4) of the protein kinase activation of the hydroxylase. These results indicate that the hydroxylase is normally maintained in a low activity state by BH_4 . When blood and tissue levels of phenylalanine are elevated, phenylalanine sets in motion a series of events which facilitate the ability of the hydroxylase to be activated by cAMP-dependent protein kinase. Once the activated hydroxylase disposes of the load of phenylalanine, the hydroxylase would return to its largely de-activated state, thus ensuring that the hydroxylase does not deplete the organism's small pool of free phenylalanine.

We have also demonstrated that phenylalanine hydroxylase is in an activated state in diabetic rats and that administration of insulin to these rats de-activates the enzyme. These effects of insulin lack and insulin sufficiency probably reflect the well-known ability of glucagon and insulin to work in opposition. These findings raise questions about what other metabolic consequences might follow from having hepatic phenylalanine hydroxylase in a chronically activated state.

In our previous studies of patients with hyperphenylalaninemia (HPA) due to defects in BH_4 synthesis, we had shown that either BH_4 or its synthetic analogue, 6-methyltetrahydropterin (6MPH $_4$), when given in proper doses, can markedly elevate brain levels of these pterins. Unexpectedly, we found that when given to two different BH_4 -deficient patients, the response was quite variable, a dramatic improvement in neurological signs being shown in only one of these children. We have now shown that there is a close correlation between the ability of the pterin to improve the neurological status of the patient and the ability of the pterin to elevate CSF levels of the metabolites of the biogenic amines. These results provide strong support for our

previous postulate that the neuropathology in these patients is due in part, at least, to a lack of tyrosine and tryptophan hydroxylase activity consequent to a generalized deficiency of BH_4 . Furthermore, our results suggest that it may be necessary to provide BH_4 or 6MPH_4 to BH_4 -deficient patients prior to birth in order to get the maximum benefit. Our results also strongly suggest that BH_4 may be necessary for normal brain development.

We also have evidence that in the patient who did not respond to pterin therapy, a more favorable response was elicited by the administration of the neurotransmitter precursors, DOPA and 5-hydroxytryptophan. These results indicate that a combination of pterin and neurotransmitter precursors may be effective in most patients.

As we have pointed out previously, the results that we have obtained from our studies of pterin therapy of HPA due to defective BH_4 synthesis have important implications for the treatment of other disorders that are believed to be caused by defects in catecholamine and serotonin metabolism, such as certain forms of depression.

In our studies of the mechanism of stretch-induced skeletal muscle hypertrophy, we have found that extracts of hypertrophied muscle contain a substance(s) that can stimulate many, and perhaps all, of the biochemical changes that are seen when muscles are stretched, including the uptake of the non-metabolized amino acid, α -amino isobutyric acid and the incorporation of phenylalanine into protein. Control muscle appears to contain detectable levels of this substance(s) but less of it than hypertrophied muscles. The identification of the nature of this material will likely help in the elucidation of the mechanism of this biological response.

Dr. Neville and his colleagues have continued their studies of the use of receptor-mediated transport to develop a new class of highly specific drugs. They have now used a mixture of anti-T cell mono clonal antibody-ricin conjugates, which they have prepared, to treat human donor marrow during bone marrow transplantation in order to eliminate graft-versus-host disease. Preliminary results have been highly encouraging. This approach should facilitate the use of bone marrow transplants in the treatment of a wide variety of diseases, such as autoimmune diseases, and other disease of the immune system such as acquired immuno deficiency syndrome.

Dr. Nash and his colleagues have continued their studies of site-specific genetic recombination, using as a model system the integrative recombination of bacteriophage lambda DNA with the host *E. coli* chromosome. They have found that the ability of prophage sites to undergo excisive recombination is critically dependent upon their relative orientation. These prophage sites cannot be recombined if they are oriented on a single circle of DNA in a head-to-head fashion nor do they recombine when they are on separate circles. From these results, they conclude that the recombination machinery must slide DNA past one site until the second site is found. By contrast, integrative recombination appears to involve random collision, rather than a sliding mechanism, in order for recombination to take place.

Annual Report of the Laboratory of Neurophysiology
National Institute of Mental Health
Edward V. Evarts, M.D., Chief
October 1, 1982 -- September 30, 1983

The goal of the Laboratory of Neurophysiology (LNP) is to advance understanding of the function of the brain as this understanding may be relevant to neuropsychiatric illness. Though several of the different NIH institutes have laboratories of neurophysiology, the research in each of these laboratories has a different emphasis. Within the NIMH LNP the special emphasis is on understanding those brain processes that are pertinent to behavior (both normal and abnormal) and to neuropsychiatric illness. LNP research workers pursue these broad goals using a number of different techniques. Indeed, within recent years the sorts of techniques that are in use in the laboratory have undergone a dramatic change. A decade ago most of the work in the LNP utilized electrophysiological techniques, but the work being carried out currently depends very heavily on the neuroanatomical and neurobiological techniques that allow discovery of the correlates between brain structure, brain chemistry, and brain function. In order to carry out research involving these new approaches it has been necessary to recruit scientists with a variety of new skills. LNP has been extremely successful in its program to bring in scientists with the necessary expertise, and of the ten positions that the laboratory devotes to scientific personnel, six are utilized for neuroanatomists. There are currently three tenured scientists in the laboratory, one (Evarts) being a physiologist, another (Herkenham) being an anatomist, and the third (Wise) an anatomist and biologist. Each of these three scientists, (Herkenham, Wise and Evarts) is responsible for one of the three major research programs of LNP.

Herkenham is responsible for a research program dealing with studies in which brain chemistry, receptor localization, brain metabolic activity, and brain development are correlated with information obtained from fiber tracing techniques using autoradiography and horseradish peroxidase. The techniques that Herkenham and his colleagues have worked out hold great promise for providing new insights into possible abnormalities of neurotransmitter receptors in a variety of neuropsychiatric illnesses.

The second major area of work in the Laboratory of Neurophysiology is carried out by Evarts and his group. This area may be referred to as Motor Psychophysics and involves studies of brain activity in subhuman primates and, in addition, studies of motor function in normal subjects and patients with neuropsychiatric disorders. Studies in subhuman primates are aimed at discovering how movements are programmed by the brain, with an emphasis on furthering our understanding of the ways in which sensory information is utilized in controlling movement. A key technique in studies of the subhuman primates is the recording of activity of individual nerve cells in many different parts of the brain. Such recording has no clinical justification in human subjects but it is possible to record muscle activity and a number of indices of movement in human subjects and by combining the information obtained from the subhuman primates with that obtained from man it is possible to make reasonable inferences as to the processes occurring in the brains of human subjects during motor performance. The purpose of this combined examination of motor control in man and monkey is to obtain a better understanding of how the brain controls movement and how disease of the brain interferes with motor control in neuropsychiatric illness.

Wise and the scientific staff members in his group are carrying out a series of projects aimed at understanding the way in which the different regions of the cerebral cortex interact in the control of movement. Whereas Herkenham and his group emphasize receptor binding techniques and fiber tract tracing, the work of Wise and his group is concerned with neurophysiological correlates of behavior and their neuroanatomical bases. The studies of Wise combine anatomical techniques with recordings of neuronal activity during movement in order to define the fields of cortex important in the cerebral control of movement and their functional specializations.

In what follows there will be a summary of the work in each of these three major divisions of the LNP research program.

I. Functional Neuroanatomy:

Work in this area is guided by Miles Herkenham and carried out in collaboration with Sandra Moon Edley and Ronald Hammer. The projects now in progress may be divided into two groups, one involving neurochemical approaches to neuroanatomy and the other dealing with metabolic correlates of brain activity. In the summary that follows these two approaches to "dynamic brain structure" will be dealt with in two parts.

Neurochemical Investigations:

For the past four years, a collaborative effort with Dr. Candace Pert in the Clinical Neuroscience Branch of the NIMH has been directed toward localizing the brain sites of action of pharmacologically active drugs and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and terminal distributions of fiber pathways in any given region. By such analysis one can identify the specific neuronal systems that contain the relevant transmitters and thereby make predictions about the neurochemical's role in normal brain function. Determinations of the locations and densities of the receptors in relevant brain regions have been used in tests of receptor changes during development, after chronic pharmacological manipulation, or in neuropathological tissues.

To permit these analyses, Herkenham and his associates have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections. Using emulsion autoradiography, the receptor distributions can be compared with underlying morphology seen in the stained sections. Stains for other features of brain chemistry and functional heterogeneity can be applied to the same or adjacent sections. Using film autoradiography, the receptor patterns can be analyzed by a densitometer for computer-assisted quantification of receptor densities.

Results from autoradiography and biochemistry together have supported the hypothesis that the high affinity mu and delta opiate receptor subtypes are different conformations of a single, interconvertible, dynamic receptor. In a similar fashion, the kappa opiate receptor subtype was characterized and visualized. Studies of the development of opiate receptors and related neurochemical systems

are in progress in an attempt to better understand the role that receptors play in the establishment of neuronal connections. Opiate receptors undergo phylogenetic changes as well, as suggested by our finding that the ratio of mu to delta receptor binding increases in parallel with "relatedness to humans" (as indicated by cytochrome C analysis of several vertebrate species). Analysis of mu opiate receptor distributions in rat and rhesus monkey cerebral cortex has revealed several general principles; e.g., opiate receptor density is greatest in limbic and polysensory (association) cortices.

These findings, taken together, suggest a role for opiates in brain function that is much more complex than previously thought and indicate that further analysis of the dynamic aspects of the receptor, after pharmacological or behavioral manipulations, might enhance our understanding of its function. Ultimately, we might hope to determine the role that opiates and related neurochemicals play in human brain function, especially in receptor-mediated mental disorders or neuropathology.

Metabolic Correlates of Functional Activity:

Taking advantage of the proximity of Sokoloff and his expertise in use of deoxyglucose to study brain metabolism, we are currently involved in developing high-resolution autoradiographic techniques for the cellular localization of metabolic activity at the light microscopic level. Patterns of metabolic activity marked by [^3H]2-deoxyglucose uptake are compared in normal, alert rats and in animals given drugs or prior behavioral experience. Using series of adjacent tissue sections from a single animal, patterns of metabolic activity during drug administration can be correlated with the localization of receptors to which the drug binds. Cryostat-cut sections are quickly dried and exposed to tritium-sensitive film to reveal the localization of diffusible [^3H]2-DG-6-phosphate, which is the major 2-DG breakdown product. Alternate sections from an animal previously injected with 2-DG can be dried at zero degrees and processed for receptor localization. This is accomplished by first removing the diffusible 2-DG in preincubation solutions prior to in vitro receptor binding. In this way, the alteration of brain metabolism by drugs or anesthetics may be correlated with receptor binding in those brain regions affected.

Analysis of 2-DG uptake in animals given anesthetic doses of ketamine has confirmed that phencyclidine-induced changes in brain metabolism occur primarily in limbic regions. Another striking variation is the relatively greater 2-DG uptake in neocortical columns, many of which are in register with granule cell-poor zones of layer IV in somatic sensory-motor cortex. The columnar patterns of uptake occur in restricted cortical zones innervated by nonspecific thalamic or intracortical fibers. The significance of this relationship of selective cortical afferent innervation and its differential activation during ketamine anesthesia is currently under investigation. Superficial layers of the entorhinal cortex and subiculum also show selective and dramatic increases in 2-DG uptake. Assuming that ketamine's effects on metabolism are initially mediated by its action at the phencyclidine receptor, such increased activity may be correlated with the patterns of tract terminations originating in regions rich in these receptors. These results suggest that phencyclidine influences brain metabolism by selectively affecting the activity within neo- and paleocortical systems.

II. Motor Psychophysics:

This second major LNP research area is directed by Evarts and involves collaboration with a number of investigators in NINCDS. Work carried on in this project has two facets. The first directed by Sanes involves studies of motor function in patients with neuropsychiatric disorders as well as in healthy control subjects and is aimed at understanding the basis for motor abnormalities in neuropsychiatric illness. The second involves study of activity of nerve cells in monkey cortical and subcortical structures. The activity of these nerve cells is observed in association with voluntary movement, and the data obtained provide information as to the mechanisms underlying control of movement by the brain.

Human Studies:

Sanes and his colleagues have recorded muscle activity and kinematics of limb position while (1) subjects manually match a target display with either a rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain postures when limb position is passively changed. Movement amplitude, presence or absence of visual feedback of position, disturbances of the subject's movements and changes in sensory input are independent variables. A second approach records a variety of psychomotor variables from patients with Parkinson's disease, cerebellar disorders, and peripheral sensory neuropathy.

The importance of afferent information in motor control is readily apparent in patients with neurological disorders such as peripheral neuropathy resulting in sensory loss, but tests of motor skills have not yet developed to the point of providing us with useful quantitative indices of the way in which afferent input is used in controlling motor output. Use of physiological techniques combined with motor psychophysics and modern computer displays, together with collaborative work with clinical neurologists, is allowing the present project to clarify a number of longstanding problems of sensory control of movement. Thus, it has been found that the postural responses of patients with cerebellar ataxia and sensory neuropathy are different from normal subjects in that there is a greater variability in the static responses for all sizes of passive displacement. For patients with sensory neuropathy the deficits appear when visual guidance is removed. This suggests that the "memory" of the motor command degrades quickly in the absence of peripheral feedback. Furthermore, the end point error of movements by patients with sensory neuropathy is extremely sensitive to preloads.

Another procedure to determine motor acuity of normal subjects and patients with neurological diseases involves determining the spatial accuracy of repetitive step movements of various sizes. Both normal subjects and patients with peripheral sensory neuropathy performed large and small movements accurately when visual guidance was available. Movement accuracy deteriorated for both normals and patients when visual guidance was absent, but the patient with sensory neuropathy showed two to four times more error than normal subjects. Furthermore, whereas the error of normal subjects decreased in absolute magnitude when smaller movements were performed, the spatial error exhibited by deafferented patients decreased only a small amount when the smallest movements were performed.

Another method to evaluate sensori-motor processes of movement has been to study the perception of muscular effort by normal subjects and patients with neurological disorders. Normal subject can detect very small changes in muscular effort,

but unimanual comparisons are superior than bimanual comparisons. Patients with sensory neuropathy, although able to detect muscular effort, are grossly impaired in their ability to detect differences between muscular efforts. The patients performance deteriorated when visual cues were removed and particularly so for bimanual comparisons.

A second facet of work in human subjects has involved development of a computerized system for obtaining objective and quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 100 patients with parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine, pergolide and nadolol. Patients with cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested. An additional use of the computer system has been to begin studies of psychomotor performance using the methods devised by experimental psychologists. These studies will be useful to provide objective measures of the organization of motor performance and to assist the clinician in diagnosis and evaluation of patients with sensorimotor disorders.

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed. Parkinsonian patients undergoing treatment with lisuride, bromocriptine, pergolide or nadolol were tested on a regular basis throughout the course of the buildup and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date has been permanently stored in computer files and is accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

These two aspects of the work in human subjects will provide clarification of how tactile and kinesthetic sensory inputs are used to control skilled motor activity, clarification that is essential to the understanding of normal and abnormal motor behavior in humans. In addition, these studies will potentially develop standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patient's progress on medication regimens is particularly suited for objective analysis.

Brain Activity During Movement: The second part of work on this project involves utilization of single neuron recording and operant conditioning techniques in

behaving monkeys to study brain mechanisms underlying voluntary movement. Cerebral control of voluntary movement depends upon the integration of internally generated central programs with peripheral inputs arising from sensory receptors. Understanding, treatment and prevention of movement disorders in neuropsychiatric patients requires knowledge of the sites at which this integration takes place. Two cerebral structures (Sensorimotor Cortex and Basal Ganglia) are of particular significance in control of voluntary movement, and current LNP work is aimed at discovering how these two structures interact. Work on the basal ganglia is of particular significance in connection with clinical disorders, since it provides techniques for discovering how toxic substances produce Parkinson's disease and how therapeutic agents exercise beneficial effects in Parkinson's disease. Work on volitionally-related activity in sensorimotor cortex provides information as to the way in which the central set and expectancy of the subject may modify processing of afferent input information and generation of output signals to spinal cord.

Striatal processing of information from the cerebral cortex is critically dependent on the neurotransmitter dopamine, and the release of this neurotransmitter is regulated by a relatively small number of cells in a part of the basal ganglia called the substantia nigra (SN). A tract of fibers (the nigrostriatal tract) links SN and striatum and activity of cells in SN causes release of dopamine in striatum. Parkinson's disease results from loss of SN cells and is accompanied by marked reductions of dopamine levels in striatum. It is not yet known how the activity of striatal cells is changed in parkinsonism, but the recent discovery that parkinsonism can be produced experimentally in the monkey by a drug (NMPTP) provides a model of the human neurological disorder in which this information can be obtained. Work thus far has demonstrated the existence of a class of tonically active striatal cells whose responses are contingent upon the behavioral significance of a sensory stimulus. This class of cells may provide set-dependent links between input and output.

A second phase of this project on cerebral control of voluntary movement has dealt with effects of volitionally initiated movement on somatosensory information processing in the cerebral cortex. In this phase of the project responses of somatosensory cortical neurons to peripheral stimuli were determined when a vibrotactile stimulus served as a cue for the initiation of an active movement executed with the stimulated extremity. It has been found that cells in somatic sensory cortex are subjected to at least two types of influences. The first occurs just after stimulus onset and is present only preceding movement of the hand. The second occurs at approximately the same time as peak precentral cortex. Both types of influences may have important central, as opposed to peripheral, components as evidenced by their dependence on and correlation with active movement.

In general terms, then, this project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex

action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum. Within the past year this project has begun a new phase involving a combined neuropharmacological-behavioral investigation of cells that depend on a dopaminergic input from the substantia nigra. Anti-parkinsonian agents act on these cells, and the availability of a new primate model of parkinsonism opens a number of new approaches to understanding the effects of dopamine in the brain. Such information is clearly relevant to the research program of the NIMH, since a major feature of many neuroleptic agents is antagonism to dopamine.

III. Information Processing in Sensorimotor Cortex:

The third major area of research within the Laboratory of Neurophysiology is being carried out by Steven Wise and his colleagues with the LNP, John Donoghue and Von Jennings.

One of the major goals of the work being carried out by Wise and his colleagues has been to obtain an understanding of the successive transformations of information leading from sensory input to motor output. Such transformations must occur, for example, when a visual stimulus located at a particular point in space is the target for a visually guided limb movement. The visual information must be used to select patterns of muscle discharge that will guide the limb towards the visual target. It seems likely that the transformations involved between visual input and motor output involve passage of nerve impulses between visual cortex and the premotor and motor cortex as well as between these cortical areas and basal ganglia. Wise has developed experimental paradigms that enable him to examine the changes in discharge patterns of neurons in the premotor, precentral motor and somatic sensory cortex during a variety of visually guided movements.

The work of Wise and his group on cortical information processing deals with

- 1) Hierarchical Organization in Motor Control Systems
- 2) Somatic Sensory and Motor Activity of Cortical Cells
- 3) Input-Output Organization in Somatic Sensorimotor Cortex

The work in these three areas involves three different research approaches: 1) The work on Hierarchical Organization has a neuropsychological orientation that is designed to provide information as to the way in which frontal lobe nerve cells control complex, highly skilled voluntary movements and the preparation for such movements. 2) The studies of Somatic Sensory and Motor Activity combine neurophysiological and anatomical techniques to identify the cell types that carry out different sorts of information processing in somatic sensory and motor areas. 3) The work in Input-Output Organization seeks to apply sophisticated electroanatomical techniques (intracellular injections of tracers into antidromically identified cells) in studies that at the same time provide information as to the behavioral and pharmacological properties of the cells. In the following summary, each of these three approaches will be considered.

Hierarchical Organization in Motor Control: One general objective of this project is an improved understanding of the organization of the entire motor cortex and its role in the cerebral control of movement. The general motor cortex is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery. The other related objective is a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

One aspect of this project dealt with premotor cortex and, in this work, monkeys were conditioned to align two spots of light on a tangent screen in front of the monkey. One of these spots is controlled by the computer (target), the other by arm movements of the animal (position). The monkey was required to align the spots within a small accuracy "window." After a short period of time the target light jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target light dimmed, at which point he was required to move (flex or extend his forearm) rapidly and accurately to the target position. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to withhold no movement. This experiment was designed to contrast neuronal activity in MI and premotor cortex during periods when a visuospatial signal instructs the monkey to perform a given movement and when identical stimuli signals the withholding of movement.

The results of this study have provided the first clear demonstration of "set-related" (in contrast to movement-related) activity in premotor cortex. It was found that whereas many neurons in premotor cortex markedly change activity before the onset of a voluntary movement, a substantial population of neurons are clearly related to planned motor activity. Several experiments have been and are being designed to further test this proposition and to elucidate the properties of these neurons under a variety of experimental conditions.

Somatic Sensory and Motor Activity of Cortical Cells: This second phase of the work by Wise and his group involves a study of the role of sensory inputs to the cerebral cortex in the control of motor behavior in primates. The first part of the project consists of a comparison of neural activity in the somatic sensory cortex (SI) and the precentral motor cortex (MI). The second part of the project is a detailed examination of the signals which the peripheral receptors are sending to the cortex during perturbation of voluntary movements. Such information should provide important clues concerning the pathway taken by peripheral inputs to MI and its role in the initiation and control of movement. This work provides an important interface with the motor psychophysics group in the LNP.

Comparisons of MI and SI in this project reveal a difference between the two areas: in SI neurons are found with a non-muscle-like relation to force and position. A muscle which is more active when the forearm is supinated is always more active when supinating force is applied to an immovable object MI cells are invariably like muscle in this regard. In SI, however, a substantial proportion of cells that are active with supinating force (without movement) became more active with the forearm pronated. The presence of both muscle- and non-muscle-like neurons in SI is consistent with that regions' postulated role in sensory processing. In order to interpret inputs which signal a mixture of position and force information it is necessary to have two inputs which code position and force in different ways. In that way two independent functions of two variables are created. In contrast, the lack of nonmuscle-like neurons in MI is

consistent with its role in motor control. MI output does not uniquely specify force or position independently. Instead it appears to specify muscle tension, which depends on both force and position.

Much work in the past year has been devoted to understanding the neural and muscular events that follow the interruption of a voluntary movement. This questions is important to the understanding of the mechanisms by which the motor and somatic sensory cortex may compensate for obstruction of intended movements.

Input-Output Organization in Sensorimotor Cortex: The third area of the work of this group is designed to examine the input-output organization of neurons in the motor cortex. Rats have been chosen as the primary experimental animal since they are widely used in the pharmacological, immunocytochemical and electron microscopic studies with which we wish to correlate our neurobiological and neuro-behavioral data. The project has four parts: (1) Neuroanatomical and neuro-physiological techniques are being employed to characterize the first motor cortex (MI) in rats and determine the connectational relationships of MI with other neural structures. (2) Single cell recording techniques are being used to record neuronal activity from several classes of projection neurons in the forelimb area of MI cortex in awake, behaving rats. In these experiments, the relationship of the neuronal activity to motor output will be examined for each type of neuron, e.g. corticospinal and corticorubral cells. (3) Intracellular recording is combined with intracellular injection of a tracer, horseradish peroxidase, to visualize the intracortical distribution of neuronal processes belonging to identified projection neurons. And (4) putative neurotransmitters of corticofugal neurons are being identified with immunocytochemical methods.

In order to antidromically identify cortical neurons sending their axons to different targets, stimulating electrodes are placed in the pyramidal tract, corticospinal tract, contralateral cortex, and in some cases, in other subcortical targets of MI neurons, such as the red nucleus. Each antidromically identified cell is injected with the tracer, horseradish peroxidase (HRP). At the termination of the experiment, the animal is perfused, the tissue is sectioned with a vibratome, and the distribution of tracer within the cell is demonstrated with histochemical techniques. Using these techniques, both pyramidal tract neurons and commissural neurons have been studied. Examination of the axonal arborizations of these cells reveals that both types of MI projection neurons have extensive intracortical connections, suggesting that they have an important role in information processing within MI as well as in sending signals to their projection targets. The transmitters of cortical neurons are identified with immunocytochemical techniques. Antibodies to synthetic enzymes of putative neurotransmitters, such as glutamate, are applied to tissue sections from rats to label neurons of specific types.

One of the major advantages of these new procedures is that they will allow anatomical data on individually identified neurons to be correlated with information on receptor localization, neurotransmitters and, ultimately, on brain metabolic activity. Thus, one aspect of the techniques that Wise and his colleagues are developing are gradually being combined with those of Herkenham and his group, while another serves to support and extend the work of Evarts and his group. The combined approaches of these three groups hold great promise for major advances in understanding of the structural, neurochemical, and physiological mechanisms of behavior.

IV. Outlook:

The foregoing sections of this summary have described three major areas of research within the Laboratory of Neurophysiology (LNP). Much of this research is newly undertaken and has involved recently recruited scientists whose skills and training equip them to take maximum advantage of the many promising neurobiological techniques that have developed in the last five years. The LNP has evolved from a laboratory that was primarily oriented to neurophysiology ten years ago to one that now combines a number of interdisciplinary neuroscience techniques in studies aimed at understanding brain mechanisms in neuropsychiatric disorders: neuropharmacology, as well as neurophysiology. This evolution is still taking place, and within the past year major progress has occurred in the coordination of research within the laboratory and the formulation of new experiments that focus on mechanisms of higher brain function. To promote this formulation Evarts and Wise have been preparing a monograph on the subject of a neurophysiology of behavior and higher brain functions under the auspices of the neurosciences Institute of the Rockefeller University, a newly established organization designed to promote innovation in neurobiological research. This project has involved two conferences aimed at identifying the most promising approaches to combined behavioral, anatomical and neurophysiological experiments in the future. It seems likely that the conceptual and experimental approaches developed in this monograph will have a major impact on research in the neurobiology of higher brain function and its completion is expected within the next year.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

October 1, 1982 - September 30, 1983

The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); and (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; and (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Branch, he first meets with the Branch Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, RSB will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Branch Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available from RSB), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The RSB does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Branch's CAN.

SECTION ON INSTRUMENTATION AND COMPUTERS

INSTRUMENTATION

The Branch has a staff of six engineers and six technicians to design, develop, and fabricate electronic and mechanical instruments. The major effort is in the production of electronic instruments for basic neurophysiological research, and for clinical studies involving affective disorders. The following are brief descriptions of representative projects, chosen from a total of 225 projects completed this year.

(1) Patient Activity Monitoring System. The Branch has continued to develop the Patient Activity Monitor (PAM) and the support hardware and software which forms the system.

(a) Monitor. The major hardware advance this year was the introduction of a new PAM which stores 10 days of data, with 15 minute resolution, and is half the size of its predecessor. The new PAM utilizes small-outline plastic packages for logic circuits, and leadless carriers for memory circuits. All parts are readily available and inexpensive. Assembly is performed by a contractor; yet the total cost of the new PAM is half that of the old device. The Branch is preparing to produce the new PAM in large quantities, with the goal of replacing all old models within the next year.

(b) Telecommunications. This project involves the development of a PAM data telecommunications system, which will allow data to be transmitted to the Branch's VAX computer via telephone, from anywhere in the country. The subject will have a microprocessor-based device in his home; it will accept the PAM, read the data, dial the phone number for the VAX, establish communication, transmit the data, clear and reset the PAM. Local software will put the data in the subject's continuous file. A prototype device is now being tested, but the software development is incomplete.

(c) Test/Initialization Instrument. In addition to reading, storing, and analyzing activity and temperature data, the PAM readout minicomputer has been used to perform several routine test and initialization procedures on the

activity monitors. Since it would often be more convenient to perform these procedures in the wards or clinics, a small handheld instrument has been developed for use with the new PAMs. Responding to pushbutton commands, the instrument can: (1) display the PAM battery voltage and indicate its condition; (2) initialize each of the 1024 PAM memory locations to a value of zero and give an error message if any location fails to initialize; (3) initialize the PAM memory address pointer to the first memory location; and (4) read and display the first 32 activity values contained in the PAM. In order to realize these functions in a portable, battery-powered instrument, a CMOS microprocessor and support chips were used.

(2) Data Acquisition System for Isolation Rooms. The design of a computerized data acquisition system for two patient-isolation rooms has been completed. This system allows the study of biological rhythms and the cyclic nature of certain mental illnesses by monitoring human subjects who are isolated from all time cues. Periodic reading of temperature and activity from the subjects are obtained through electrically isolated signal conditioners. These data values, along with average room illumination readings, are converted to serial digital data and transmitted over a two-wire link to a minicomputer storage and display system. The data are reformatted into the PAM continuous file structure and on-line hard-copy of the three measured parameters is generated. A second design phase will incorporate touch-input CRT terminals for patient mood self-ratings and patient-staff communications.

(3) EEG Amplifier System. The design has been completed and construction begun on a new 32-channel EEG amplifier system. The design incorporates several new integrated circuit components and printed circuit board layouts to permit construction of a compact, low cost-per-channel unit. The system will be used for several ongoing research projects including topographic brain mapping. The simplified construction and small size will permit expansion to a 64-channel system in the near future.

To facilitate the use of the EEG system in varying experiments and on different computers with different A/D input requirements, the unit was made as flexible as possible. For example, switched-capacitor filters were used, in which the cut-off frequency is controlled by the frequency of an input clock. The computer can then control the bandwidth of the amplifier system by changing the clock frequency.

Sample-and-hold modules are also incorporated in the design to prevent "skewing" errors associated with sampling a large number of channels. The sampling frequency can also be varied as needed, and front panel switches allow the user to switch the filters or sample-and-holds in or out as desired.

The 32 data channels are also available as a 16 channel multiplexed output for use with the Grass polygraph.

(4) Discriminator and Iontophoresis Systems. Five RSB amplitude/time window discriminator systems were completed this year. The use of CMOS logic and the recently redesigned printed circuit boards allow the units to be versatile and reliable. The versatility of the design allows each unit to be tailored to the needs of the requestor. Several discriminators were built with tone generators, and one included a special post stimulus, adjustable time window. In addition, a printer interface designed around a small Datel printer is available.

Also, two modified units for processing post-synaptic potentials were completed this year. Four RSB 5-channel iontophoresis systems were also completed and are currently being used in neuropharmacological studies in the IRP.

(5) Neurophysiological Data Preprocessor. A microprocessor system is being developed to replace the custom logic circuitry used by the present Laboratory of Neurophysiology Data Acquisition System. The new preprocessor will record the times of occurrence of 64 different events and 8 different pulses. This information will be transmitted to the central processor through a parallel interface and the information will be coded in such a form as to ensure compatibility with the present software that is used for analysis and display of the data. The preprocessor will decrease response time to events and pulses, and free the central processor for experiment control.

(6) Neuro-PET Scanner Chair and Gantry Controller. A gantry and electro-mechanical chair controller was designed and installed into the Neuro-PET Scanner, which was developed by NINCDS in conjunction with BEIB. The controller has facilitated the positioning of eighty-one patients in the ring of the scanner. The design of the controller includes a digital readout of the exact position of the head of the patient, and various safety stops to ensure patient and machine safety.

(7) Visual Evoked Response Stimulus System. A visual evoked response stimulus system has been built, that will randomly select one of eight 35 mm slide images and project it on to a 35 cm x 50 cm opaque screen. The projection system uses a very fast electromechanical shutter (2.3 msec opening time) for a fast rise time in presenting the image. The slides are mounted on a circular disc, which is rotated by a direct-drive stepper motor. The maximum random access time for any slide is 125 msec. The stepper motor is controlled by a special purpose processor that can be linked to either a computer or a terminal through a standard RS 232 serial interface.

(8) Conference Room Microphone System. The versatility of the Building 36 conference room public address system was increased by implementation of an audio summing amplifier interface that allows up to 12 inexpensive lapel microphones to be used around the conference room table. The lapel mikes were also adapted for use by speakers using the podium.

(9) Four-Arm Radial Rat Maze. An elevated multi-level, 4-arm radial rat maze was designed, constructed, and installed to assess the effects of neuropeptides on learning, memory and perception in laboratory rats. Audible and/or visual cues are presented at the end of a randomly selected arm. The path of the animal is monitored by detectors located at selected positions throughout the maze. When an animal traverses the proper path to the cues, a programmable liquid reinforcement is dispensed. At the end of the testing period, statistical data is printed regarding the animal's performance. An eight-bit microprocessor single board computer is used to monitor and control the maze and perform the statistical calculations.

COMPUTERS

Small computers are ideally suited for laboratory research in neurophysiology and psychology. They are used in the laboratory for on-line, real-time interactions, process control, and data acquisition. Recorded data may be stored,

combined with other data, reduced statistically, transferred to larger computers for further analysis, transformed for presentation graphically or mathematically, and the results may be printed or plotted. Increasing use is being made of the small computer for processing the text of scientific papers and communications. Data base management is now available for the small computer, as are limited management information systems.

Techniques have been developed for image processing which are applicable to many diverse experimental systems, ranging from autoradiographs of brain tissue sections to the analysis of two-dimensional electrophoresis gels.

Larger mini-computers, the so called super-mini's, have been reduced in price and are now available for functions formerly performed by larger time-shared systems. These systems allow applications in modeling, curve fitting and statistical treatment that would be prohibitively expensive on large systems.

Inexpensive personal computers are proving useful for dedicated applications. Many scientists are developing software for these computers, which they offer to the scientific community at minimal cost. They will become increasingly useful in the laboratory and their potential is currently being studied.

Microcomputers are used in the design of biomedical instrumentation. This provides a savings in design and fabrication time for instruments and often provides a more flexible system than one based on discrete components.

The Research Services Branch is actively involved in the applications of small computers in the IRP. By integrating the functions of biomedical instrument design and laboratory computer systems with software designed specifically for the research community, the Branch offers computer support services for a broad range of scientific disciplines.

LABORATORY COMPUTERS

The design goal for the laboratory instrument computer is to provide maximum function, tailored to the specific experimental design, with minimum cost. RSB provides consultation on the specification and selection of laboratory computers for new applications; conducts systems studies in collaboration with the scientist; and helps the scientist in the procurement, installation and maintenance of the equipment.

To help in these efforts, RSB maintains two support computers, one in Building 36, and one in the Clinical Center. These systems provide the more expensive equipment necessary for off-line data storage, efficient data processing, communications with DCRT computers, and plotting and printing of the data. The systems are run on an open shop basis and are used for program development, training, and testing the feasibility of new systems for the laboratories.

TRAINING AND SOFTWARE SUPPORT

RSB provides training for the scientist or support personnel who will be programming and maintaining the system. Personnel limitations make it impossible for RSB to provide applications programming, so such programming must be supplied by the laboratory. RSB computer personnel are always available for consultation,

training, and help in debugging, as well as assistance in the selection of part-time programmers or consultants. Commercial software packages or applications from other research labs are often available, and RSB will evaluate such systems.

RSB maintains a library of procedures which were written specifically for the laboratory computers used in the intramural community. These procedures are designed to be incorporated into the users' programs. In addition, RSB will aid the investigator in writing the difficult time and data dependent sections of real-time programs.

PERSONAL COMPUTERS

Personal computers have the potential to become powerful and useful laboratory computers. RSB is conducting a study of the different models for future use in the laboratory. Manufacturers are offering increasingly sophisticated word processing packages, and they may replace some of the commercial word processors now being rented. Other software packages provide data base management, management information systems, and spread sheet accounting.

In the near future, hardware and software should be available to incorporate these systems into a network, tying them into the larger computers of the IRP. Integrating these systems will provide a cost effective utilization of equipment for laboratory and administrative personnel.

PROGRAM MAINTENANCE

There are now more than 60 minicomputers in the program; many of these systems have been in use for years. The programs used on these systems were written by a number of people, many of whom are no longer in the IRP. Design of these programs is such that changes are usually required as the experimental protocol develops, so program maintenance is a continual, and time consuming function of the Branch. Structured programming techniques and standardization of equipment have enabled the Branch to provide these services without an increase in personnel.

MICROPROCESSORS

The Branch maintains a microprocessor development system for the software and hardware development of microprocessor-based instrumentation at both the chip and single board computer level. The system currently supports three common microprocessors: one 16-bit processor, and two 8-bit processors.

IMAGE PROCESSING SYSTEM

The RSB maintains a general purpose image processing system consisting of an Optronics rotating drum film scanner, a DeAnza image array processor, and a PDP-11/60 computer. Images to be processed may be obtained by scanning autoradiographs, x-ray film, or photographic negatives, or by using images generated by CAT or ECAT scanners. A camera station is available to generate color hard-copy using Polaroid SX-70 or 35 mm film.

Interactive, menu-driven, software packages have been developed to provide an extensive and expandable repertoire of basic image processing functions.

Special purpose functions can be developed to meet specific user requirements. The facility is useful for numerous applications involving evaluation and quantification of biomedical images. The two primary applications of the system are the densitometric analysis of autoradiographs of brain or tissue sections and the analysis of two-dimensional electrophoresis gels. The software currently available on the PDP-11/60 based system is being converted to run on the multi-user VAX-11/750 computer which is managed by the Branch.

VAX COMPUTER SYSTEM

The Branch manages a multi-user VAX-11/750 computer system that is available for use by all investigators in the IRP. The VAX is located in Building 36, in space furnished by the Laboratory of Cerebral Metabolism, NIMH. Potential users in Building 36 may request installation of hard-wired cable connections, or the VAX may also be used on a dial-up basis.

The primary use of the VAX is for image processing applications. A high resolution color image display is now operational and an Optronics film scanner directly connected to the VAX will be available in the near future. In addition, an Ethernet link is being developed to allow high-speed transfer of images between the VAX and the existing PDP-11 based image processing system.

A device-independent graphics package has been developed on the VAX that permits plots to be generated on numerous display terminals and hardcopy devices. A terminal emulation program is available which permits small laboratory computers to function as graphics terminals when using the VAX, as well as supporting file transfer in both directions.

The SPICE2 circuit analysis program has been installed on the VAX and is being used by several investigators for modeling neuronal circuits. Programs have been written to generate graphical displays and hardcopy plots of the output of the SPICE2 program.

OTHER APPLICATIONS

(A) Neurophysiological Data Analysis System. This system was initially developed for the Laboratory of Neurophysiology, NIMH, and has found widespread use. It is a versatile system, relating neurophysiological data such as neural events or the EMG with behavioral events related with learning, discrimination, perception, etc. The system provides visual presentation of the data, relating all events in time. The system was extended to allow further selection from the data, sorting the data on criteria derived from analysis of time dependent areas of the data, and further statistical analysis of the data. An improved data acquisition system is currently being designed; this will require rewriting the data acquisition programs. The resulting system will enable the investigator to add elements utilizing the computer in the control of the experiment and presentation of the stimuli.

(B) Rat Motion Cages. The rat motion cages developed by the Branch for the Adult Psychiatry Branch, NIMH, will be used to measure epileptic seizure activity in rats. The animals will be maintained in the cages for long periods of time to measure the effectiveness of proposed treatments on the diminution of seizure activity.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care is staffed by a veterinarian, laboratory animal technicians, and animal caretakers who provide daily care and maintenance of laboratory research animals. Services provided by the Section include:

(1) Management of the Central Primate Facility. The Central Primate Facility has a capacity for approximately forty primates. At the present time, three investigators have research projects in progress. Support is provided through assistance in preparing surgical models, insertion of catheters, and maintaining animals on special diets and feeding routines. The Section has also developed the capability for bacteriological culturing and identification, pH and blood gases, and non-invasive blood pressure monitoring.

(2) Tuberculosis Testing Program. All primates, including those located at St. Elizabeth's Hospital, are tuberculosis tested every three months.

(3) Treatment of sick and post-operative animals.

(4) Procurement of all animals for the Division of Intramural Research, NIMH.

(5) Providing day to day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all areas. The supervisor of the Section provides day to day contact with personnel in all work areas and coordinates weekend and holiday schedules. Night coverage of the primate nursery continues to work very smoothly with two part-time persons covering on alternating nights. Also, these two persons administer night medications to animals on treatment.

(6) An orientation program is being developed for new investigators and technicians who will be working with laboratory animals. It should be implemented in the fall. Subjects to be covered include animal care support, housing of animals, availability of animals, and special diets and feeding.

(7) Animal facilities. Upgrading and additions to the animal holding facilities continue. Renovations and additions to Building 9 are in progress and scheduled for completion in October, 1984. Cages are being purchased for the new animal room in Building 10. The fourth floor of the ACRF has been turned over to the Institute, but due to inadequate ventilation only a limited number of animals can be housed there.

Although the animal holding facilities at St. Elizabeth's Hospital and Poolesville are not part of the Section due to logistic difficulties, the veterinarian is responsible for the quality of animal care delivered. Consultant visits are made periodically to assist in care and maintenance of research animals and to take care of health problems that may exist.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Branch's workload among the various laboratories and branches.

<u>LABORATORY OR BRANCH</u>	<u>HOURS</u>	<u>PERCENT</u>
Clinical Psychobiology, NIMH - - - - -	4513	14.54
Biological Psychiatry, NIMH - - - - -	3614	11.66
Neurophysiology, NINCDS - - - - -	3044	9.81
Clinical Science, NIMH - - - - -	2761	8.97
Neurophysiology, NIMH - - - - -	2359	7.60
Psychology and Psychopathology, NIMH - - - - -	2231	7.19
General and Comparative Biochemistry, NIMH - - - - -	1467	4.73
Cerebral Metabolism, NIMH - - - - -	1410	4.54
Neuropathology and Neuroanatomical Sciences, NINCDS - - - -	1370	4.42
Adult Psychiatry, NIMH - - - - -	1254	4.04
Neuropsychology, NIMH - - - - -	887	2.86
Surgical Neurology, NINCDS - - - - -	865	2.79
Neurochemistry, NINCDS - - - - -	831	2.68
Molecular Biology, NINCDS - - - - -	674	2.17
Biophysics, NINCDS - - - - -	486	1.57
Experimental Therapeutics, NINCDS - - - - -	390	1.27
Neurochemistry, NIMH - - - - -	343	1.11
Molecular Genetics, NINCDS - - - - -	232	.75
Neural Control, NINCDS - - - - -	179	.58
Neuro-Otolaryngology, NINCDS - - - - -	171	.55
Developmental Psychology, NIMH - - - - -	107	.35
Clinical Neuroscience, NIMH - - - - -	76	.25
Central Nervous System Studies, NINCDS - - - - -	63	.20
Infectious Diseases, NINCDS - - - - -	58	.19
Neuroimmunology, NINCDS - - - - -	57	.18
Service on Committees - - - - -	923	2.97
NIMH (Total)	21,125	68.08
NINCDS (Total)	8,392	27.04
NICHD (Total)*	1,512	4.88
	31,029	100.00

*NICHD loans the Branch one position, and is thus entitled to 1700 hours of service.

ANNUAL REPORT OF INTRAMURAL PROGRAM AT
SAINT ELIZABETHS HOSPITAL, NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1982 to September 30, 1983

Another busy--and fruitful--scientific year in the NIMH Intramural Program at Saint Elizabeths Hospital is coming to a close. Looking back over the past year, I see a settling down period after a time of much change. I am most relieved and pleased to say that our program has weathered the changes well and we have continued to produce excellent science throughout. Credit goes to the strong leadership of Drs. Herbert Pardes and William Mayer as well as the conscientious efforts of all our scientists and staffs.

The Intramural Research Program, newly under the direction of Dr. Frederick Goodwin, is stabilizing. The Division structure was abandoned with the intention of increasing a coordinated leadership. Rather than each Division being run as a separate entity, the plan was to run the Intramural Program with an enhanced sense of interaction. I am adjusting to my new roles and the administrative changes appear to be positive.

But changes never stop occurring and I regret the departure of Dr. Irwin Kopin. Dr. Kopin has been Chief of the Laboratory of Clinical Science for many years, and more recently the Associate Director for Basic Research. What appears to be our loss, however, is the gain of the National Institute of Neurological and Communicative Disorders and Stroke. I wish him great success as that Institute's Scientific Director and look forward to much continued interaction--both personal and scientific.

With great pleasure, however, I warmly welcome the return of Dr. Seymour Kety to the Intramural Program as the Associate Director of Basic Research. I look forward to working closely with him in the months and years ahead.

Just as we are saying hello to Dr. Kety, we must say good-bye to our Administrative Officer, Carl Pergler. Carl has been with the program at Saint Elizabeths Hospital for eight years. During his time here he has greatly improved the safety and appearance of the William A. White Building. This is evidenced by the high marks received from the JCAH. Although we continue to have funding problems modernizing the facility, I hope this, too, will be corrected in the not-too-distant future. In the meantime, Carl's efforts have gone far in improving the building's working conditions.

While excellently managing our Program, he has also greatly improved our relationship with Saint Elizabeths Hospital. The most recent example is that he has succeeded in formalizing a much needed agreement with the Hospital. We now have a signed agreement placing support services on a cost reimbursable basis. We hope that with this arrangement our clinical support staff will be stabilized and that facility services will be more reliably performed. Carl is owed our deepest thanks for this and his many other accomplishments. I wish him much success his new position with the David Taylor Model Basin. He will be sorely missed.

I want to make mention of Dr. Wallace Mendelson's move from the Adult Psychiatry Branch here at Saint Elizabeths to the Clinical Psychobiology Branch, Unit on Sleep Studies at the main NIH campus. He started his long affiliation with us while a medical officer at Saint Elizabeths Hospital and has now become a leader in the field of sleep research. Also, I would like to wish Donald Hinton the best in his retirement and to Emil DeRenzo much success in his new position at the Mitre Corporation in McLean, Virginia. My final good-byes

are to Dr. Darwin Cheney who has taken his new post at the Ciba-Geigy Corporation in Summit, New Jersey and to Dr. Aurora Revuelta as she moves to the Mount Sinai School of Medicine as Chief of the Laboratory of Gas-Chromatography, Mass-Spectrometry in the Department of Psychiatry.

An example of the highlights of this past year's accomplishments in the Laboratory of Preclinical Pharmacology is Drs. Guidotti's and Wise's and Mr. Konkell's success in purifying, to homogeneity, a peptide from rat brain with 129 amino acid residues. This peptide, GABA-modulin (GN), appears to be located in synaptic membranes. Guidotti's team's work indicates that GABA-modulin acts as a coupling factor in the reciprocal interaction between GABA and benzodiazepine recognition sites.

Also over the past year, Dr. Chuang has established a laboratory for the preparation of monoclonal antibodies. This new laboratory has already produced monoclonal antibodies to the beta-adrenergic recognition site for catecholamines. These antibodies provide the necessary tools to develop a cDNA for the beta-adrenergic recognition site.

A final example of the excellent work performed by Dr. Costa's group is Drs. Yang's and Lindberg's investigation of the preproenkephalin A. These researchers have been exploring how preproenkephalin A is processed to a final putative neuromodulator. Drs. Yang and Lindberg are purifying and characterizing the enkephalin generating enzyme previously isolated from bovine adrenal gland.

The Adult Psychiatry Branch, as well, has achieved much over the past year. I am extremely pleased by the progress of Dr. Luis de Medinaceli's nerve repair research and the continued success of our grafting work. I am further impressed that Dr. Farouk Karoum and his colleagues in our Mass Spectrometry Unit have performed close to 10,000 assays. Dr. Daniel Weinberger and colleagues have performed approximately 115 studies with our new cerebral blood flow equipment, and this work continues to expand.

Together the Program has enjoyed over 65 speakers lecturing on a wide range of topics, and over 150 papers have been published. Guest workers from Argentina, China, Egypt, France, Greece, Italy, Israel and Japan have given the William A. White Building a truly international flavor. We enjoy the cross cultural mix. Also, we want to congratulate Dr. Ermino Costa on his Troy C. Daniels Lecturer Award from the University of California at San Francisco as well as Dr. William Freed for his A.E. Bennett Award for his transplantation work.

The final two matters to address are the remnants of last year's RIF and the question of a new building for our programs on the main NIH campus. As for the former, of those employees bumped into our program, only one now remains. We hope that all those who have been resettled are comfortably and appropriately situated. The RIF put a great deal of pressure on those individuals and they conducted themselves well during their predicament. As for the latter point, plans for a new building for FDA and our program on the NIH grounds were submitted this past spring. Current estimates call for the construction specifications to be completed sometime in FY 1984. Timing of construction is unclear due to present budget constraints.

In closing, I would like to say again that this past year has been an important and an eventful one. Our programs, laboratories and leadership have sustained substantial change. To the credit of all, we have squarely faced the changes, met the challenge and appear

stronger and more productive than ever. It gives me great pleasure to be able to report so positively this year; and I look forward to reporting even better news next year.

ANNUAL REPORT OF THE ADULT PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1982 to September 30, 1983

Richard Jed Wyatt, M.D., Chief

In the Adult Psychiatry Branch our interests span the fields of neurochemistry, neuropsychology, neuropharmacology, neuroanatomy and neurophysiology, especially as these disciplines influence research on neuropsychiatric mechanisms and disorders and in particular the schizophrenias and the problems of the aged. Because psychiatry must incorporate a number of subspecialties in the investigation of mental health and mental illness, the Adult Psychiatry Branch synthesizes the talents of many physicians and researchers from various branches of scientific medicine.

Scientifically, the 1982-1983 reporting period has been another excellent year. Dr. Kleinman's collection of autopsied brains has grown from over 200 to over 300 in the past year. This collection has become an important resource to our researchers as well as researchers from other laboratories. Also, using a postmortem approach Drs. Emino Costa, Ingeborg Hanbauer, and Joel Kleinman demonstrated that the dopamine sensitive adenylylase activity is increased in the nucleus accumbens and caudate nucleus of chronic schizophrenic patients compared with controls and, although our CT scan has been presenting some problems, our cerebral blood flow research is underway.

In the area of clinical assessments, two innovations first mentioned in last year's Annual Report have continued to progress. The first is the Premorbid Adjustment Scale (PAS) developed by Ms. H. Eleanor Spoor and Dr. Steven Potkin. The PAS addresses the problems of conceptualizing premorbid adjustment. Premorbid adjustment is defined as the attainment of certain developmental goals (those viewed most frequently as necessary milestones for healthy functioning) and the attainment of those goals as specific age-related tasks. It was created to evaluate, at each of several periods of a subject's life, the level of functioning in four major areas. The scale is intended to measure only premorbid functioning and its reliability and validity were tested on various healthy and ill populations. Over the past year, the PAS has been sent to clinicians around the country. Preliminary data are currently being collected. Also, Dr. Lynn DeLisi and her colleagues, in a study designed to collect historical and outcome data on schizophreniform disorder patients, found that poor premorbid adjustment as assessed by PAS correlated positively with poor outcomes. This work was a follow-up study of previous research in which CT scans had been performed. The DeLisi work further showed that the poor premorbid patients were those patients with large ventricles.

The second assessment innovation, spearheaded by Dr. Llewellyn Bigelow and implemented by Mr. Ivan Waldman, was the development of a computer program to allow online entry of behavioral data directly from our three inpatient units. The program enables the rater to respond to serially presented questions regarding each patient. The program also permits rapid correlations of behavioral, physiological and biochemical data.

In work looking for markers for schizophrenia, Dr. John Morihisa, collaborating with Dr. Frank Duffy at Harvard University, has continued over the past year to apply the technique of brain electrical activity mapping (BEAM) to the study of our patients. Their findings continue to show an increased amount of frontal delta wave activity in unmedicated chronic schizophrenic patients.

Although our drug abuse research is being phased out, our work, with the collaboration of Dr. Elizabeth Parker, NIAAA, has continued in the area of alcohol use. Over the past year, this research has continued to produce interesting results. Dr. Parker's work indicates that certain memories are strengthened by alcohol consumption depending on whether the alcohol is consumed before or after the learned task has taken place. These findings were supported by Mr. A. Paul Oliver's work with hippocampal slices. In this work, Mr. Oliver showed that in animals having drug induced seizures that there was enhancement or decrease in seizure (spike) activity depending on when the animal was given alcohol. In other related work, Drs. Bruce Phelps and Parker have been investigating a possible relationship between monoamine oxidase activities and alcohol consumption. This work will be reported in a future Annual Report. Finally, Drs. Peter Bridge and Parker have begun testing older subjects to assess drinking patterns of elderly normal adults.

In our Aging Program, our work exploring Alzheimer's disease continues to progress. Dr. David Shore's work investigating the aluminum-fluoride complexes has focused on the use of fluoride to complex aluminum already absorbed in an individual animal's system. Results of these preclinical studies using small samples showed that fluoride treated animals tended to have lower aluminum concentrations in the brain. Dr. Shore has continued, also, with his therapeutic study of Alzheimer's patients to study whether or not the progressive deterioration of this disease can be slowed by oral administration of sodium fluoride. In this longitudinal study, he is also investigating the degree and type of language impairment in the disease. Finally, Dr. Shore is working to develop a test of lymphocyte vulnerability to aluminum toxicity.

Turning to the disorder of tardive dyskinesia, Dr. Dilip Jeste has developed a set of diagnostic criteria. These criteria include multiple descriptions of the phenomenology, various historical aspects pertinent to TD development, and characteristics of treatment response. Further, given the increased risk of developing TD by older patients, we have developed a set of guidelines for using neuroleptics with elderly patients.

Dr. Weinberger has investigated the previous finding of large cerebral ventricles, in some schizophrenic patients, in an elderly population. Dr. Weinberger's work has pursued the questions: 1) Might the CT findings described in young schizophrenic patients also be found in elderly patients? 2) What impact does aging have on these CT findings? and 3) Is cerebral atrophy caused by many years of treatment? As for the first question, results showed that advancing age was associated with large ventricles. As for the second question, results were consistent with previous research; cerebrospinal fluid-filled spaces of the brain increase in size with age. In response to the final question posed, results of this work indicate that the effects of prolonged institutionalization and drug treatment on ventricular size are minimal.

Dr. de Medinaceli's work on nerve repair has progressed well during the past year. He has developed an index based on the measurements of the footprints of walking rats that provides a reliable and easily quantifiable method of evaluating the functional condition of the animals' reconnected sciatic nerve. Results of this method, the sciatic functional index (SFI) provide a very precise measure. Large amounts of data are now being computer-quantified by a program developed by Mr. Emil DeRenzo.

Last, and anything but least, we look at our brain grafting work over the past year. Our initial work with rats has continued to grow and expand. For this work, Dr. William Freed won the A.E. Bennett Award this year. The grafting has progressed to the stage of rhesus monkeys and, while this advancement in level of complexity has presented its own

new obstacles, the monkey work is beginning to produce positive results. Also, we have been refining our histochemical techniques and are challenging immunocompatibility boundaries.

As in the past, this reporting year's work is the result of the cumulative efforts of an extraordinarily talented group of scientists and support staff. Some are new to our laboratory and some have been with us for many years. Using this space for what is becoming an annual opportunity for me to say thank you to many people for their excellent work, I also want to say a special hello and good-bye to our newcomers and our departing staff. This past year we have been joined by Urmi Patel, Frank Putnam and our new Staff Fellows Haim Bracha, Jack Grebb, and Richard Shelton. We welcome Drs. Lavadre and Garg from India for the summer and our Visting Guest Workers Yen Nung Wang from China, Renaud de Beaurepaire and Anne Marie Duchemin from France. We are happy to welcome Steven Potkin back from the Beijing Medical Center in Peking and want to offer our congratulations to Drs. William Freed, Joel Kleinman and Daniel Weinberger for their appointments to tenured status. Our congratulations to Dr. William Freed for receiving the A.E. Bennett Award and I am pleased to tell you that I received the Stanley R. Dean Award as well as being counted as one of the The Washingtonian Magazine's Future Leaders of Washington. Certainly we shall have to await the outcome of that prediction!

As we say hello to some, we must say good-bye to others. Those we are saying farewell and much luck to are T. Peter Bridge, as he takes his new post at Parklawn. We say good-bye to Dr. Michael Sherer who moves to the Clinical Psychobiology Branch and to Dr. Richard Wagner. We also say good-bye to Dr. Wallace Mendelson and his staff of Cindy Owen, Joseph Martin, Wendy Pinckney and Ruth Timmons. Finally, as I look back over this past year, my assessment is for a year of high achievement, energy and progress for the Adult Psychiatry Branch. I look forward to reporting the same, this time next year.

Annual Report
Laboratory of Preclinical Pharmacology
October 1, 1982 thru September 30, 1983

The discovery that two or more molecular species of putative neuromodulators coexist in the same axon has generated a number of new research strategies. For a pharmacologist, it has become important to evaluate which strategies offer the best perspectives for the development of a much needed new generation of centrally active drugs. These drugs need to be more effective and to have a better safety margin than those drugs presently available. These strategies require new working models for pre- and postsynaptic mechanisms. Presynaptically, we need to study whether the turnover rate of a given monoamine and a given peptide that coexists in a given axon changes simultaneously and whether these changes are in the same or in an opposite direction. While techniques to measure monoamine turnover are available, techniques to measure neuropeptide turnover are still in disarray. In evaluating neuropeptide dynamic state, it must be considered that they are synthesized and incorporated into high molecular weight precursors which are packaged in synaptic vesicles with the appropriate enzymes. Each of these precursors includes either many different neuropeptides or several copies of the same peptide. It is therefore impractical to use incorporation of labeled amino acids into the peptide to measure neuropeptide biosynthesis unless one has the capability to separate and measure the specific activity of the immediate precursor of the terminal molecular form of the putative cotransmitter. Since such a separation is practically impossible currently the dynamic state of the neuropeptides is assessed by measuring the steady state of the neuropeptide and the amount of specific mRNA. If both are increased, it is probable that the turnover is increased.

Postsynaptically, we are convinced that the development of new drugs will be facilitated by an increased understanding of the supramolecular organization of receptors regulated by multiple synaptic signals. Among these chemical signals there are some basic differences: some are cotransmitters, others are transmitters. Transmitters are chemical signals that can be transduced into a stimulus for the postsynaptic cell, cotransmitters are those signals that cannot be transduced but modulate the gain at which the transducer operates.

In the postsynaptic working model currently followed in this laboratory, receptors are viewed as supramolecular entities including one or more recognition sites for multiple specific effectors. These sites are linked by a pertinent coupler device to an enzyme or an ion specific pump which transduce the chemical signals into metabolic stimuli for the postsynaptic cells. A transducer can either be directly coupled to a primary transmitter recognition site or be indirectly regulated by one or more cotransmitter recognition sites.

Past experience suggests that it is dangerous to repair the consequences of pathological abnormalities of synaptic transmission by chronically using drugs that block or activate primary transmitter recognition sites. Usually, synaptic function cannot be permanently activated or inhibited without experiencing important changes in collateral synaptic functions leading to untoward side effects.

Because this recurring sequence of events appears to be triggered by every drug known to act on primary transmitter recognition sites, our current research strategies in receptor research are directed toward the identification of cotransmitters which appear to be ideal target sites for drug development. We believe by acting pharmacologically on the

cotransmitter mechanism we can develop useful tools for future clinical investigation with greater efficacy and safety than those presently available.

Two novel strategies are currently being pursued to identify new cotransmitters. One consists of identifying those endogenous effectors that act on high affinity recognition sites for drugs which do not act on primary transmitter recognition sites. These drugs have a low level of risk with regard to untoward side effects (benzodiazepines, imipramine, etc.). The other consists of identifying a functional link between a primary transmitter and a neuropeptide cotransmitter by showing synaptic interdependence with appropriate histochemical, physiological and pharmacological techniques. Once the interdependence is characterized, appropriate agonists or antagonists are synthesized and used.

When developing concepts to follow up these new research strategies, we were helped by our studies on the mechanism of action of benzodiazepines which have led to the identification of a novel brain neuropeptide that functions as a putative cotransmitter for GABA-ergic mechanisms and to the characterization of GABA-modulin, a new coupler mechanism functioning on GABA recognition sites following its phosphorylation and dephosphorylation. During this year, the achievement of many research objectives was fostered by the on-going acquisition high technology methodology related to recombinant DNA, to monoclonal antibodies and to ion channel studies. Presently, Dr. Neff is setting up a laboratory of patch clamping to study molecular regulation of transmitter activated ion pumps.

Section on Neuroendocrinology (Chief, A. Guidotti, M.D.)

I. Pharmacology of GABA-ergic synapses

The close functional and anatomical association between GABA and benzodiazepine binding sites raises the question of whether the site that recognizes GABA at postsynaptic GABA receptors is an oligomeric structure or whether it is a complex of entirely separated proteins; one recognizing GABA, the other recognizing endogenous effectors whose function is that of modulating GABA recognition sites, Cl^- ionophore interactions. One of the sites where such an endogenous effector (cotransmitter?) acts is the site where benzodiazepines bind. In order to understand GABA receptor function, we decided to isolate, purify, and characterize some of the proteins that function in mediating the interactions between GABA, benzodiazepines and the Cl^- ionophore complex.

Drs. Guidotti, Wise (PRAT Fellow) and Mr. Konkel have been successful in purifying to homogeneity from rat brain a peptide with 129 amino acid residues termed GABA-modulin (GM) which is located in synaptic membranes and acts as a coupling factor in the reciprocal interaction between GABA and benzodiazepine recognition sites. GM is a basic peptide because it is rich in arginine and lysine. When added to a synaptic membrane preparation it prevents specifically the binding of ^3H -GABA or ^3H -muscimol to specific high affinity recognition sites located in synaptic membranes and also prevents the diazepam induced increase in the B_{max} of muscimol binding.

In addition to a large number of basic residues the GM molecule contains a relatively large quantity of serine residues which are preferential phosphate acceptor sites when located in amino acid sequences with which have an optimal affinity for protein kinases. This observation prompted a series of experiments in which Dr. Wise was able to show that GM was extensively phosphorylated by cyclic AMP and Ca^{2+} -dependent protein kinase and that GM phosphorylation by cAMP dependent protein kinase but not by the Ca^{2+} -dependent

enzyme resulted in a complete loss of GM capacity to inhibit high affinity ^3H -GABA and ^3H -muscimol binding.

This regulation of GM function through phosphorylation was observed using a reconstituted system. This observation led to an investigation of whether GM is associated with GABA receptors and whether GM can be phosphorylated in synaptosomal membranes without the addition of exogenous cAMP dependent protein kinase. Dr. Wise was able to show that membrane bound GM was phosphorylated when synaptic plasma membranes were incubated with cAMP, Ca^{++} and Ca^{++} plus calmodulin. These results suggest that in synaptic membranes the necessary phosphorylating enzymes are located in proximity to GM and may, in fact, be functionally related to the GABA/benzodiazepine/ Cl^- ionophore receptor complex.

Dr. Vaccarino (Guest Worker), using brain synaptosomes obtained by flotation sedimentation sucrose gradient method was able to show that GM is a natural constituent of the synaptosomal membranes and its removal from the membranes results in an increase of ^3H -muscimol binding. Dr. Vaccarino's work has also shown that GM is a basic protein with some chemical similarities to myelin basic proteins but it differs from the latter because of amino acid composition, carboxy terminus group, subcellular distribution and ability to inhibit high affinity binding of GABA to synaptic membrane.

Dr. Novelli (Guest Worker) has initiated a purification of the benzodiazepine recognition site located in brain synaptic membranes. The benzodiazepine recognition sites were photolabeled with ^3H -flunitrazepam, and a rapid procedure was developed for the separation of the 55,000 MW subunits of the benzodiazepine binding sites. This peptide is currently being purified and monoclonal antibodies for this recognition site are being prepared. We plan to use these antibodies to purify mRNA for the benzodiazepine recognition complex. This probe will allow us to recognize the sequence of the benzodiazepine recognition sites.

Drs. Guidotti and Corda (Guest worker), with the assistance of Mr. Konkel have continued the characterization of the endogenous effector of benzodiazepine binding sites. Following the observation that crude extracts from brain inhibited beta-carboline and benzodiazepine binding we proceeded to isolate, purify to homogeneity and partially sequence a peptide (DBI=diazepam binding inhibitor) which prevents the binding of ^3H -benzodiazepine and of esters of ^3H -beta-carboline-3-carboxylic acid to synaptic membranes. Experiments carried out with the collaboration of Dr. Bennett (Merck Sharpe and Dohme, West Point, PA) have established that DBI contains 104 amino acid residues and the sequence of 45 amino acid residues, and does not resemble any sequenced mammalian peptide. The brain recognition site for benzodiazepines can bind various anxiolytic compounds with an affinity proportional to their pharmacological potency, but it also binds benzodiazepine analogues practically devoid of intrinsic activity and esters of beta carboline 3 carboxylic acids endowed with proconvulsant and or anxiogenic activity.

There are some specific binding properties that distinguish the binding of the three classes of compounds. Tested in a battery specific tests, DBI behaves like the esters of beta-carboline-3-carboxylic acid and can be differentiated by benzodiazepines. In order to test the effect of DBI in vivo, Dr. Corda has developed an in vivo behavioral test whereby it is possible to differentiate anxiolytic benzodiazepines from anxiogenic esters of beta-carbolines-3-carboxylic acids in rats exposed to a conflict punishment situation. Using this model, Dr. Corda has been able to show that DBI given intraventricularly and the esters of beta carboline 3 carboxylic acid given parenterally block the anticonflict action of diazepam. Further, by themselves, DBI and the esters induce punishment responses which are blocked by an antagonist of benzodiazepine receptor such as the derivative RO 15 1788.

We are now investigating whether DBI is a precursor of a smaller molecular weight peptide. In preliminary experiments, we have obtained evidence that the biological activity resides in one of three fragments that are obtained by cleaving the polypeptide at the methionine residue with CNBr. Moreover, we are also ascertaining whether brain contains only anxiogenic rather than anxiogenic and anxiolytic effectors.

Another approach that we have taken to elucidate the molecular mechanisms operative in the *in vivo* interactions at these receptors, has been to develop *in vivo* binding models to study the down regulation and supersensitivity of receptors. Two goals we intend to pursue are: 1) to obtain basic information on the validity of *in vivo* and *in vitro* binding studies and 2) to obtain models that can be used in man for the application of gamma or emission computed tomography scanning to the study of brain transmitter receptors. Along this line of investigation Dr. Ferrero (Guest worker) has already demonstrated that it is possible to measure ^3H -muscimol specific binding *in vivo* using rat and mice. In addition, diazepam, but not the esters of beta-carbolines-3-carboxylic acid produce a definite change in the *in vivo* characterization of ^3H -muscimol binding. This change may reflect a benzodiazepine induced allosteric alteration of the binding of endogenous GABA to the high affinity recognition sites. This alteration was responsible for the increase of GABAergic transmission induced by benzodiazepines *in vivo*. Thus, the GABA/benzodiazepine interactions were confirmed in *in vivo* studies.

11. Pharmacology of the interaction between cotransmitters (opiate peptides) and primary transmitters (catecholamines and acetylcholine) in the adrenal medulla.

The synapses between splanchnic axon terminals and the chromaffin cells are known to contain nicotinic cholinergic receptors for acetylcholine (ACh) stored in splanchnic axons. Recent evidence indicates that in splanchnic axons ACh may coexist with enkephalin like immunoreactive material. It has been hypothesized that nerve impulses with specific characteristics of frequency can release simultaneously enkephalin like peptides and ACh, and that when these neuroactive substances are coreleased they may interact by stimulating their specific recognition sites located on the chromaffin cell membranes. Hence, specific recognition sites for ACh and opioid peptides may be part of a supramolecular unit that controls nicotinic receptor function.

Dr. Saiani (Visiting Fellow) has characterized the opiate recognition sites of adrenal medulla and she has concluded that they cannot be classified as recognition sites, but instead, belong to a novel type that specifically binds etorphine and Met-enkephalin-arg-phe. These two opioid ligands are the most potent in inhibiting ACh-induced release of catecholamines from primary culture of bovine adrenal by cells. To prove that the opiate peptides stored in the splanchnic nerve participate in the modulation of nicotinic receptors, Dr. Saiani and Dr. Hanbauer (NIHLB, Bethesda) measured in anesthetized dogs the effect on the catecholamines released from adrenal of electrical stimulation of the distal end of splanchnic nerve severed below the diaphragm. Diprenorphine, a blocker of the opiate receptors, facilitates the secretion of catecholamines elicited by electrical nerve stimulation. These results prompted Dr. Saiani and Dr. Kageyama (Guest Worker) to initiate an investigation on the molecular mechanisms of the interaction between opiate and nicotinic recognition sites. In order to relate changes in ACh recognition sites to opioid cotransmitter action, various toxins contained in various commercial sources of alpha-bungarotoxin were studied. Using the venom of bungarotes multicinctus, Dr. Saiani and Dr. Kageyama were successful in isolating a toxin termed P4 bungarotoxin, which is different from alpha- or beta-bungarotoxin, and selectively and competitively blocks the release of catecholamines from chromaffin cells elicited by ACh. This ACh action cannot be blocked by either alpha or beta-bungarotoxin. Dr. Kageyama is now exploring whether P4 bungarotoxin can be radiolabeled and used as a ligand to study the nicotinic recognition

sites in brain or medullary cells. Our ultimate goal is to study the molecular mechanisms that are operative in nicotinic function modulation by opiates in adrenal medullary cells.

Since some adrenal medulla cells contain catecholamines and enkephalin-like material in the same granules, Drs. Kageyama and Kataoka (Visiting Fellow) have begun an investigation aimed at identifying the stimulus characteristics related to the material stored in the granules that contain more than one chemical signal. Moreover, since in chromaffin cells, protracted stimulation of membrane receptors also causes long term modulation of catecholamines and proenkephalin biosynthesis, they are studying how this modulation is mediated. So far, available data suggest that different mechanisms are involved in the modulation of the release and biosynthesis of catecholamines and enkephalins evoked by acetylcholine.

In chromaffin cell cultures, 8-Br-cAMP (0.1 to 1 mM) induces after 2-3 days an increase in the number of proenkephalin mRNA and tyrosine hydroxylase (TH) and enkephalin molecules; moreover, the cyclic nucleotide increases the release of the enkephalins. All this information taken together suggests that 8-Br-cAMP activates enkephalin and TH biosynthesis. The increase of both proteins is prevented by actinomycin D or by colchicine treatment. The latter must be added before the cytosolic protein kinase activated by the nucleotide translocates from the cytosol to the nucleus. Since colchicine prevents nuclear protein phosphorylation by inhibiting translocation of catalytic subunits of protein kinase from the cytosol to the nucleus, it is believed that in chromaffin cells, phosphorylation of nuclear proteins is involved in controlling gene expression of mRNA coding for TH and proenkephalin. We believe that these studies in primary cultures of adrenal medulla cells may represent a useful model for studying how gene expression of primary transmitters and cotransmitters is controlled during long term adaptive changes.

Section on Molecular Neurobiology (Chief: E. Costa, M.D.)

I. New Technology

Progress in this section was fostered by a methodological renovation brought about by the introduction of new recombinant DNA and immunological techniques. During this reporting period, Dr. Chuang has established a laboratory for the preparation of monoclonal antibodies. He has obtained monoclonal antibodies to the beta-adrenergic recognition site for catecholamines; thus, providing a necessary tool to obtain a cDNA for this protein. A partially purified beta-2 adrenergic receptor recognition site from frog erythrocytes was used as the antigen to immunize mice (balb/c) and the spleen cell of these immunized mice were further immunized *in vitro* with the antigen. These spleen cells were fused with myeloma cells (P3x63 Ag 8), a line secreting Ig G, K chains. These cells were screened using the immunoprecipitation of ¹²⁵I-iodohydroxybenzypindolol labeled solubilized beta-adrenergic receptors. Positive cells were cloned by limiting dilution to obtain monoclonal cultures and antibodies were produced by growing hybridomas as ascite tumors in mice.

The monoclonal antibody produced precipitated beta-2 adrenergic receptors solubilized from membranes of frog erythrocytes and from rezeptosomes formed during isoproterenol-induced desensitization of the frog erythrocyte membrane receptors. The immunoprecipitation curves of the two preparations differed suggesting that this monoclonal antibody has a lower affinity for the internalized beta-receptors. The immunogenic site to which this antibody binds is distinct from the ligand binding site of this receptor.

Another line of investigation pursued by Dr. Chuang concerns the measurement of mRNA content coding for insulin-like peptides present in brain tissue (Exp. Cell. Biol. 143:

351, 1983). Using a cDNA probe for rat proinsulin prepared by Dr. Villa Komaroff, Dr. Chuang is studying whether the mRNA coding for insulin present in brain resembles that of the pancreas and whether the brain mRNA is regulated transsynaptically.

11. GABA_A and GABA_B Molecular Organization

Dr. Majewska (Visiting Fellow) continued the study on the regulation of GABA_A and GABA_B recognition sites located in crude synaptic membranes of rat brain. She found that at 37°C, binding of [³H]-GABA to GABA_B recognition site is dramatically stimulated by Ca²⁺ with a K_a of 10⁻⁷ M while the binding of [³H]-GABA to GABA_A recognition sites is only slightly enhanced by Ca²⁺ (K_a 5x10⁻⁷ M). Only GABA_A recognition sites are linked to benzodiazepine recognition sites; moreover, the increase in the B_{max} of high affinity GABA_A recognition sites elicited by benzodiazepines is modulated by a low M Ca²⁺. Dr. Majewska labeled the phospholipid content of C6 glioma cells by preincubation with [¹⁴C]-arachidonic acid. The addition of GABA, muscimol and THIP to these labeled cells increases the degradation of phosphatidylcholine and phosphatidylethanolamine by phospholipase A₂. Concomitantly the release into the medium of [¹⁴C]-arachidonic acid and its radioactive metabolites is increased. These effects are blocked by bicuculline but are unaffected by picrotoxin and pentetrazol, which also block GABA receptors, but act on the Cl⁻ channel. Specific GABA uptake blockers, such as beta-alanine and nipecotic acid, fail to change the muscimol-induced release of arachidonate. Thus, muscimol induced elevation of phospholipid metabolism in C6 glioma cells is mediated by GABA receptors which presumably do not use a Cl⁻ ionophore as a transducer. Diazepam, flunitrazepam, medazepam, and midazolam (but not clonazepam) potentiate the effect of muscimol on arachidonate release. Moreover, this potentiation can be antagonized by R0 15 1788 but not by esters of beta-carboline-3-carboxylic acid. This suggests that coupling of diazepam to GABA recognition sites involves a type of benzodiazepine recognition site that differs from the classic one. Analysis of the radioactive arachidonate metabolites released by muscimol reveals that when the activation of GABA_A recognition site occurs in the presence of diazepam, a substantial amount of the arachidonic acid released is converted to prostaglandin D₂.

111. Mode of Action of Antidepressants

Dr. Barbaccia (Visiting Fellow) continued her studies on the role of high affinity imipramine binding sites in the regulation of 5HT uptake in CNS. Her previous results showed that in the brain of rats repeatedly treated with desipramine and imipramine, the down regulation of beta-adrenergic receptors (decrease in the density of binding sites and attenuation of the amplification of beta-adrenergic receptor-coupled adenylate cyclase elicited by NE can be prevented by a selective destruction of 5HT axonal terminals where the high affinity imipramine binding sites are located. This finding suggests that imipramine recognition sites function as binding sites for physiological effectors and that they are not drug acceptors. Moreover, while a protracted administration of imipramine or desipramine decreases the density of imipramine binding sites in the hippocampus and other brain areas, the uptake of [³H]-5HT by hippocampal minces is increased while neither the uptake of dopamine nor that of NE changes. These results clarify the function of imipramine binding sites suggesting that they are effector sites for an endogenous mechanism that controls the uptake of 5HT in a negative manner. Moreover, an intact serotonin axon terminal is essential for the down regulation of beta-adrenergic receptors and, perhaps, for the therapeutic effects of antidepressants.

Dr. Barbaccia is working on the identification of a brain component that selectively displaces [³H]-imipramine from its binding sites located in crude synaptic membranes and inhibits the uptake of [³H]-5HT by 5HT neurons. This endogenous ligand for imipramine

binding site can be extracted from brain with 95°C acetic acid, hydrochloric acid and formic acid. Since the biological activity of this effector is pronase resistant, it may not be a peptide. Chemically, it is not 5HT, tryptamine or kynurenine. We have obtained its partial purification by HPLC and we are now working on the mass fragmentographic identification of its chemical nature.

Crude synaptic membranes of rat brain also contain specific high affinity binding sites for the atypical antidepressant mianserin. It was previously suggested that ^3H -mianserin labels 5HT₂ receptor recognition sites because this binding can be effectively displaced by spiperidol and ketanserin. Recent evidence obtained by Drs. Barbaccia and Gandolfi (Guest worker), however, has shown that the number of ^3H -mianserin binding sites increases following a lesion of 5HT axons with 5,7-dihydroxytryptamine whereas the binding characteristics of ^3H -ketanserin remain unchanged. Moreover, repeated daily injections of imipramine decrease the specific binding of ^3H -ketanserin but not that of ^3H -mianserin to crude synaptic membranes prepared from rat hippocampus. These results suggest that ^3H -mianserin binding sites and 5HT₂ recognition sites may interact allosterically. Dr. Roth (Guest Worker) has initiated the identification of the endogenous effector for ^3H -mianserin recognition sites.

In collaboration with Drs. Gandolfi and Barbaccia, we have also studied the mechanism of action of two atypical antidepressants, iprindole and bupropion. Repeated daily injections of iprindole decrease the density of beta-adrenergic receptor binding sites and NE sensitive adenylate cyclase activity in the frontal cortex. However, this iprindole-elicited event is unaffected by a lesion of 5HT axonal terminals, suggesting that the site of action of iprindole may be located in the postsynaptic neurons of the 5HT synapses. A long term administration of daily doses of iprindole decreases the number of ^3H -ketanserin and ^3H -mianserin binding sites in the frontal cortex and hippocampus but fails to modify the binding characteristics of 5-HT₁ recognition sites. Experiments are in progress to determine whether iprindole effects on ^3H -ketanserin and ^3H -mianserin binding require the function of 5HT axon terminals.

Bupropion was considered to be a typical antidepressant because it was reported by others that this drug on chronic treatment, failed to modify the function of beta-adrenergic receptors. Recent studies by Drs. Gandolfi and Barbaccia showed, however, that in the brain of rats treated with bupropion (50 mg/kg, twice daily) for three weeks, the density of beta-adrenergic receptor recognition sites and the activity of beta receptor coupled adenylate cyclase are both attenuated. The specificity of this bupropion effect is supported by the finding that a dose of bupropion given twice daily for several weeks fails to change the binding of ^3H -ketanserin, ^3H -5HT and ^3H -mianserin to crude synaptic membranes. Currently, we are studying the molecular mechanism whereby bupropion effects B_2 -adrenergic receptor function.

IV. Technology for Specific mRNA Studies

Over the past year, Dr. J. Schwartz and F. Tang (Guest Worker) have set up the methodology needed to measure specific mRNAs by using a cDNA probe. Using a probe which codes for most of the gene for human pheochromocytoma preproenkephalin, which was a gift from Dr. E. Herbert and M. Comb (University of Oregon), we have shown the presence of a very similar mRNA about 1400 bases, in rat striatum, hypothalamus, cortex, cerebellum, midbrain, brain stem and hippocampus. These results suggest that the brain precursor for the enkephalin family of neuropeptides (Met-enkephalin, leu-enkephalin, met-enkephalin-arg-leu and met-enkephalin-arg-gly-leu) is very similar in structure to the adrenal medulla preproenkephalin, and further, the rat is very similar to human.

Daily doses of haloperidol repeated during the 2-3 week period elicited a four fold increase in the preproenkephalin mRNA content of rat striatum and a two fold increase in the content of midbrain but no change in any other brain region. Similar results on the specific mRNA content of striatum were obtained by Dr. Mocchetti (Guest worker) one week after a single reserpine treatment. Also, in this case the enkephalin like immunoreactivity increased two fold. These results suggest that the action of dopamine on some neuronal receptors (D_1 , D_2 or both) may exert a tonic inhibitory influence on pre- and proenkephalin synthesis because when the dopamine receptor occupancy is impaired by either haloperidol or reserpine the enkephalin content increases concomitantly with the content of specific mRNA for proenkephalin. Hence, the measurement of mRNA by this hybridization procedure allows us to begin the study of peptide biosynthesis at the level of the regulation of gene transcription.

Bovine adrenal chromaffin cells maintained in culture offer a useful model system to study molecular mechanisms that regulate the specific gene transcription Dr. Quach, (Visiting Associate). These cells express the mRNA for preproenkephalin, a treatment with 8-Br-cyclic AMP (0.1 mM or higher concentrations) increases the proenkephalin mRNA content of these cells. This increase is both dose- and time-dependent and it is not replicated by 8-Br-cyclic GMP. This increase is associated with a corresponding increase in the cellular content and secretion of high and low molecular weight forms of enkephalin like peptides.

Previous studies had shown that the addition of 8-Br-cyclic AMP induces tyrosine hydroxylase in these cells by triggering a cascade of chemical events which include translocation of the catalytic subunits of cytosolic cyclic AMP dependent protein kinase into the nucleus and the phosphorylation of proteins associated with nuclear chromatin. The necessity of translocation was documented by showing that a dose of colchicine that blocks translocation of protein kinase also inhibits the TH induction. This effect has a time requirement suggesting that neurotubules and neurotubular protein function are necessary for this translocation. Using the cDNA probe for preproenkephalin, we will now be able to study the molecular mechanisms that regulate the expression of this specific gene in the adrenal medullary cells.

V. Nerve Growth Factor

Another approach being used by Dr. Schwartz to study changes of the substance P neuropeptide content of the spinal cord is through the use of animals that form antibodies to nerve growth factor (NGF). Animals exposed in utero to anti NGF antibodies lose both sympathetic and sensory neurons. These ganglia contain a number of neuropeptides including substance P and somatostatin, as expected rats exposed in utero to NGF antibodies, the content of these two peptides in the dorsal root ganglia and peripheral tissues they innervate is drastically decreased. When adult animals are exposed to antibodies to NGF the ganglia and peripheral tissue content of the above mentioned peptides is decreased, but the neurons remain intact. Dr. J. Byrd (Guest Worker) is investigating how NGF exerts its biological effects in the PC12 pheochromocytoma cell line which is a target cell for this growth factor. Currently, he is investigating the biochemical mechanism whereby NGF can be internalized and is attempting to relate NGF internalization to its biological activity.

Recent evidence suggests that there are several molecular forms of nerve growth factors, each presumably specific for a specific population of either CNS or PNS neurons. Since Dr. Schwartz had shown previously that stimulation of beta-adrenergic receptor stimulates the synthesis of NGF from a C6 glioma cell line, we have used a neurologically deficient mutant strain of mice to study whether CNS astrocytes might similarly produce a

NGF. In the PCD mutant, Purkinje cells develop normally but die between days 20 to 50 during which time there is an active proliferation of aster glial. Mouse cerebellum contain a NGF like factor which is immunologically cross reactive with the new clonal antibodies against NGF, but has no biological activity: the content of this NGF like protein increases in PCD mouse cerebellum as neurons degenerate.

VI. Coupling, Organization, and Interactions of DA Receptors

Several types of cell culture as well as membranes prepared from striatum have been used to study the interactions of various receptors for neurotransmitters at the molecular level. Dr. P.L. Onali (Visiting Associate) has shown that dissociated cultures of rat anterior pituitary have provided a system examining the dopamine D-2 receptor. Vasoactive intestinal peptide (VIP) stimulates both adenylate cyclase and prolactin secretion from the mammothrophs of the anterior pituitary. Dopamine can block both of these responses through an action at the D-2 receptor. Through experiments with GTP, GPP-NHPP and cholera toxin, we conclude that the inhibitory coupling of the D-2 receptor with the adenylate cyclase occurs at the level of the GTP binding protein (Ni). Since there is a GTPase associated with this protein, we are currently investigating the role of GTPase in the regulation of adenylate cyclase activity by Ni.

Muscarinic receptors in the striatum are coupled to adenylate cyclase in an inhibitory manner. Occupation of these muscarinic receptors by agonists results in stimulation of a high affinity GTPase, suggesting that the activation of the GTPase is coupled to the inhibition of adenylate cyclase elicited by muscarinic agonists. The GH 3 cell line provides a uniform cell population to study this question further. VIP stimulates both adenylate cyclase and increases cyclic AMP content and prolactin secretion in these cells: acetylcholine blocks all of these responses in a comparable dose dependent manner. These results suggest that acetylcholine exerts its inhibitory influence on prolactin secretion through an inhibition of adenylate cyclase. This control occurs in the presence or absence of stimulatory hormones, such as VIP.

VII. Multiple Forms of Opiate peptides

Preproenkephalin A contains within its peptide sequence four copies of Met⁵-enkephalin (ME) and one copy each of Leu⁵-enkephalin (LE), Met⁵-enkephalin-arg⁶-phe⁷ (ME-RF) and Met⁵-enkephalin-arg⁶-gly⁷-leu⁸ (ME-RGL). From available data from this and other laboratories, it can be inferred that enkephalinergic neurons produce multiple molecular species of putative neuromodulators. Thus, in order to assess the question of the multiplicity of chemical signals, Dr. Yang has determined the distribution of ME-RF and ME-RRL in CNS, and of peripheral tissues, and then compared the results with the distribution of ME.

Dr. Iadarola (PRAT Fellow) and Dr. Lindberg (PRAT Fellow) found that ME-RGL in spinal cord and brain parallels closely with the distribution of ME and ME-RF. In addition to the octapeptide, ME-RGL, a considerable quantity of high molecular weight forms of ME-RGL immunoreactivity (MW about 8000) was detected in spinal cord, medulla pons and hypothalamus but not in striatum. Interestingly, in bovine adrenal medullary chromaffin granules more than 80% of the ME-RGL immunoreactivity was contributed by the high molecular weight form. Both authentic octapeptide, ME-RGL, and its high molecular weight form can be released from spinal cord by perfusing the subarachnoidal spaces with substance P (10^{-6} M). This result suggests that in spinal cord also the molecular weight form of ME-RGL may be a final cleavage product of preproenkephalin A functioning as a putative neuromodulator. On the other hand, the different ratios of high molecular weight form vs. authentic ME-RGL may suggest that the rate of ME-RGL formation differs in different CNS

regions.

In order to explore how preproenkephalin A is processed to the final putative neuromodulator, Drs. Yang and Lindberg are continuing the purification and characterization of the enkephalin generating enzyme previously isolated from bovine adrenal gland. This enkephalin generating enzyme extracted from bovine adrenal chromaffin granules was purified 1000-fold by combination of affinity chromatography and gel filtration. This partially purified enzyme was further characterized using various substrates such as small fluorogenic peptides, peptide E, peptide F, 8.6 K dalton Met⁵-enkephalin containing peptide (Met-enkephalin is at the C terminal) and beta-lipotropin. The small fluorogenic substrates were hydrolyzed very poorly in comparison with peptides E and F. The Km for 8.6 dalton Met⁵-enkephalin containing peptide is much smaller than that for fluorogenic substrates. Finally, beta-lipotropin was not a substrate of this adrenal enzyme. These results suggest that the enzyme isolated from bovine adrenal gland may participate in processing preproenkephalin A stored in adrenal chromaffin granules.

In order to reach a better understanding of how the action of opioid putative neuromodulators is terminated, it was of interest to study the specific enzymes involved in the catabolism of endogenous opioid peptides and to study how selective inhibitors for these enzymes change the endogenous steady state of various peptides. Previously, we found in vitro that ME-RF can be readily cleaved by a dipeptidyl carboxypeptidase in a manner that is inhibited effectively by captopril. The physiological relevance of this reaction has been studied by Dr. Chou (Visiting Fellow) following the perfusion of the rat subarachnoidal spaces. Drs. Chou and Tang (Visiting Fellow) have observed that ME-RF and ME can be released from spinal cord when the subarachnoidal spaces are perfused with substance P. The recovery of the ME-RF released by substance P was increased greatly by the addition of captopril (10^{-6} M) to the perfusion fluid but was increased only slightly by the addition of bestatin. The release of ME was prolonged by bestatin whereas thiorphan increased it only slightly. These results suggest that the ME-RF and ME released by substance P is metabolized by a dipeptidyl carboxypeptidase and an aminopeptidase, respectively. Thus, the specific inhibition of the metabolism of one of these two opioid peptides may provide a tool to study the physiological responses elicited by the corelease of these two opioid peptides that coexist in the same nerve terminal.

In the tissues of molluscs, ME-RF often exerts actions which are opposite of those of phe-met-arg-phe-NH₂ (FMRF-NH₂). Since Dr. Yang and her collaborators have found that ME-RF and FMRF-NH₂ are highly localized in the posterior horn of rat spinal cord, they studied the possible interactions between ME-RF and FMRF-NH₂ in the spinal cord. FMRF-NH₂ injected intrathecally antagonizes the analgesic effect elicited by ME-RF given intrathecally in combination with captopril. In addition, Dr. Tang showed that morphine analgesia can also be antagonized by FMRF-NH₂. A characterization of the endogenous FMRF-NH₂-like immunoreactivity present in spinal cord revealed that the bulk of the FMRF-NH₂ immunoreactive material has a chromatographic profile different from authentic FMRF-NH₂. Because of this, endogenous FMRF-NH₂ immunoreactive material was partially purified from bovine brain and the biological properties of this endogenous material was studied.

When the endogenous FMRF-NH₂-like material was injected intrathecally, it reduced the analgesic effect of ME-RF. Thus, in addition to cholecystokinin (CCK) (Facis et al., Science 219: 310, 1983) other endogenous peptides also antagonize morphine analgesia. The antagonistic action of FMRF-NH₂ and CCK against morphine analgesia indicates that FMRF-NH₂ and CCK may physiologically interact with endogenous opioids and peptides. Perhaps, such an interaction may be operative in the development of morphine tolerance.

Because of the availability of proglumide, a specific antagonist of the peripheral effects of CCK, Dr. Yang and her collaborators could investigate whether CCK modulates the onset of morphine tolerance. Dr. Tang showed that intrathecal morphine releases CCK from spinal cord. Moreover, if rats were treated repeatedly with morphine with or without proglumide, the development of acute morphine tolerance was greatly reduced in the rats receiving morphine and proglumide.

These results suggest that endogenous CCK may play an important role in morphine tolerance. Previously, Dr. Yang and her collaborators detected ME-RF, bombesin, and substance P in the rat lung and Dr. Panula (Visiting Fellow) showed immunohistochemically that ME-RF is located in small APUD-like cells around the wall of some bronchi while substance P and bombesin like immunoreactivity are located in nerve fibers close to bronchi or blood vessels.

This distribution prompted the study of the release of ME-RF from superfused lung cubes prepared from rats. It was found that ME-RF can be released from lung cubes by the addition of substance P or bombesin (10^{-6} M) to the superfusion fluid. This process is Ca^{2+} dependent. The results suggest that when substance P and bombesin are released from their neuronal storage sites they can release ME-RF from APUD cells, directly or indirectly.

Since ME and ME-RF are stored in the same striatal cells Drs. Chou and Tang studied the regulation of ME-RF striatal storage by dopamine. In rats receiving daily injections of haloperidol for three weeks, like the striatal content of ME, also that of ME-RF was increased. Following intraventricular captopril, the accumulation rate of ME-RF was greater in haloperidol than in saline injected rats. This greater accumulation rate of ME-RF supports the view that haloperidol increases the pre proenkephalin biosynthesis. This possibility is in line with the increase in specific mRNA content already discussed in this report.

Dr. Panula studied the location of ME-RGL-like immunoreactivity with an antibody specific for this opioid octapeptide derived from proenkephalin A. In rat brain this peptide was found to be distributed similarly to ME-RF-like immunoreactivity. Numerous cell bodies were found in the olfactory tubercle, neostriatum, amygdala, throughout the hypothalamus and in several pontine and medullary nuclei. MERGL was found in nerve fibers and terminals throughout the brain. Hence, MERGL is widely distributed throughout the brain and its location in nerve terminals further justifies studies on its role as a neuromodulator. These results are in keeping with the view that enkephalinergic neurons contain at least four putative neuromodulators and support the possibility that multiple chemical signals are operative in mediating transmission in enkephalinergic synapses.

In the pituitary gland, MERP, MERGL and ME-like immunoreactivity had a similar location in the posterior lobe, whereas only MERP was found in the intermediate lobe where it was colocalized with beta-endorphin. Dr. Panula concluded that a MERP-like peptide may have a role in endocrine function of the intermediate lobe.

Motilin like immunoreactivity was located in neurons of the arcuate nucleus. The visualization of where this immunoreactivity was completely abolished by preabsorption of the antisera with motilin. In cerebellar Purkinje cells, a weak staining with motilin antiserum was seen but these images did not disappear to preabsorption with motilin. Dr. Panula suggests that the chemical nature of this motilin-like immunoreactivity in these two brain areas is different.

Antisera raised against histamine were used to locate histochemically histamine containing neurons in rat brain. Numerous immunoreactive neurons were found in posterior

basal hypothalamus. The method may be a useful tool to study the brain histaminergic system. The comparative distribution of gastrin releasing peptide (GRP) and substance P in rat hypothalamus was also studied. While the terminal fields showed overlapping of the two immunoreactivities, nerve cell bodies with GRP or substance P immunoreactivities were found in different hypothalamic nuclei. It is concluded that two peptides do not coexist in the same neuron and that the neuronal systems containing these peptides in the hypothalamus are separate.

Section on Biochemical Pharmacology (Chief, N.H. Neff, Ph.D).

During the past year the section on Biochemical Pharmacology has continued studies on synaptic biochemical vents that are activated during synaptic transmission. Emphasis has been placed on synapses that contain receptors for catecholamines, adenosine, acetylcholine and GABA_B recognition sites.

I. Adenosine Receptors

Dr. Wojcik (Staff Fellow) has continued his characterization of adenosine receptors in various brain structures. The general scheme that has emerged suggests that some similarities exist between adenosine and prostaglandin. In fact, both classes of compounds lack specific storage mechanisms in tissue and appear to be formed at a rate which is appropriate to the utilization rate. Adenosine is released from slices of neostriatum by high potassium in a Ca²⁺-dependent manner. Local injections of kainic acid into the neostriatum one week before a perfusion study abolish the release of adenosine from the slice and also destroy adenosine A₂ receptors. Apparently, the elements that release adenosine as well as the adenosine A₂ receptors are present within the neostriatum, perhaps, associated with intrinsic interneurons.

While evaluating the possible interaction of other putative transmitter substances with the adenosine A₁ receptor mediated inhibition of cerebellar adenylate cyclase systems Dr. Wojcik discovered that the GABA_B receptor was also negatively coupled to adenylate cyclase. GABA and its chemical congener (-) baclofen inhibited basal adenylate cyclase activity of various brain regions by 40%. Among the various rat brain regions studied, the striatum and cerebellum displayed the greatest responsiveness to baclofen. Baclofen inhibition was insensitive to bicuculline, a specific GABA_A antagonist and was not affected by diazepam. Dr. Wojcik also observed, using neurologically mutant mice, that GABA_B receptor similarly to adenosine A₁ receptor is associated with cerebellar granule cells. In subsequent studies, Dr. Wojcik established that there is only one maximal response to adenosine and GABA_B receptor stimulation. This implies that these two receptor systems apparently share the same nucleotide binding protein (NS) and/or catalytic unit. The baclofen studies are of particular interest because baclofen is the most effective compound available for treating spasticity.

II. Photo Affinity Labeling

Dr. Cavalla (Visiting Fellow) is synthesizing photoaffinity ligands with high specific radioactivity for the adenosine receptors. The ultimate goal of this work is to have the ligand covalently bound to sites located in the vicinity of the specific recognition sites of the receptor. Hence, the ligand could become a tool to isolate and characterize the adenosine receptor. Already Dr. Cavalla has synthesized several compounds, the most active being 2-azidoadenosine. A sodium deoxycholate solubilized preparation of cerebellar crude synaptic membranes appears to irreversibly bind the compound when activated with ultraviolet radiation. Dr. Cavalla will attempt to isolate and characterize the ligand binding site(s).

III. Cyclase and Dopamine Receptor Regulation

Drs. Olanas and Onali (Visiting Associates) continued their studies on the interactions of muscarine and dopaminergic receptors coupled to a common adenylate cyclase complex in the rat striatum. They found that chronic stimulation of muscarinic receptors, achieved by administration of the acetylcholinesterase inhibitor diisopropylfluorophosphate, resulted in a decrease of basal adenylate cyclase activity of about 50%. Moreover, the ability of muscarinic receptors to inhibit dopamine activation of adenylate cyclase was completely lost. Hence, the down regulation of muscarinic receptors releases dopamine dependent adenylate cyclase from muscarinic modulation. One might consider that this could be an interesting model to explain the functional supersensitivity of dopaminergic function that is proposed to be operative in certain psychoses.

IV. Neurotransmitters and Retina

Previous work in this section has established that dopamine amacrine cells of retina are activated when rats are exposed to light. Dr. Economou (Guest Worker) found that the retinal content of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) could be a functional role of a specialized population of amacrine cells that store dopamine. When animals are placed in the light these amacrine cells are activated and DOPAC content increases; in contrast, when the animals return to the dark the cells return to a basal state of activity and the DOPAC content decreases. While completing the program, Dr. Economou observed that rat retina contained small quantities of norepinephrine and epinephrine. By removing the superior cervical ganglion, she established that norepinephrine was associated with sympathetic neurons that innervate the retina; perhaps, on blood vessels while epinephrine was stored in neurons intrinsic to the eye. Interestingly, the retinal epinephrine varied with photic stimulation being higher in the light.

In collaboration with Dr. Cohen (Assistant Professor, Department of Pharmacology, Howard University) we studied whether a norepinephrine methylating enzyme was present in the retina and found that phenylethanolamine-N-methyltransferase (PNMT) activity similar to that found in other tissues was present also in the retina. Drs. Economou, Panula and Mariani (NEI) using immunohistochemical techniques established that PNMT-like immunoreactivity is present in retina and is associated with a subclass of amacrine cells that appeared to intermingle with the processes of the dopamine-containing amacrine cells.

Reports in the literature suggest that alpha-2 adrenergic binding sites are present in retina. Dr. Economou, therefore, studied the possible interaction of epinephrine-amacrine cell with the dopamine-amacrine cells by administering alpha agonist and antagonist drugs to rats either in a light or dark environment. Alpha-2 antagonist drugs activate the dopamine amacrine cells in the dark as evidenced by an increase of DOPAC. Alpha-2 agonist drugs were inactive in the dark, but were capable of decreasing the DOPAC content in the light. We suggest that epinephrine amacrine cells actively inhibit the dopamine amacrine cells in the dark via alpha-2 receptors. The dopamine amacrine cells may be activated in the light because of disinhibition of the epinephrine containing cells. In support of this notion, the metabolism of dopamine cells can be suppressed by administering alpha-2 agonist drugs to light-exposed rats.

V. Dopaminergic Neurons in Periphery

There is now an abundance of experimental evidence to suggest that dopaminergic neurons are present in peripheral tissues. Peripheral blood vessels are especially interesting because they contain dopamine receptors that when stimulated increase blood flow to some organs and lower blood pressure. Dr. Economou has provided strong pharmacological

evidence to support the presence of dopaminergic neurons innervating the cardiovascular system. From a systematic study of the content of norepinephrine and dopamine in the cardiovascular system she found that arteries contain 5 to 8% of their norepinephrine as authentic dopamine. In contrast, the veins contain only 1 to 3 percent. Following the administration of the neurotoxin 6-hydroxydopamine both norepinephrine and dopamine fell beyond the limits of detection.

If desipramine is administered before 6-hydroxydopamine to protect the noradrenergic neurons, the dopamine content is decreased by a large extent while the content of norepinephrine is unchanged. The percentage of the dopamine content in the arteries of rats receiving 6-hydroxydopamine and desipramine approaches the content of the veins. If the specific noradrenergic neurotoxin DSP-4 is administered, only the arterial content of norepinephrine falls while the content of dopamine remains unchanged. These results are in keeping with the hypothesis that there are peripheral dopaminergic neurons in addition to noradrenergic neurons that innervate arteries. The veins would not receive such an innervation.

VI. HPLC Methods for ACh Turnover

Dr. Potter (Fogarty International Fellow) has used a HPLC method she developed to measure acetylcholine content of various structures of the rat spinal cord. A high energy microwave oven modified to focus primarily upon the spinal cord is used to kill the animals. Acetylcholine was found to be rather uniformly distributed along the cord in both white and gray matter, between 200-300 pmol/mg protein. Transection of the cord does not result in a major change of acetylcholine above or below the lesion. This is in contrast with catecholamine and serotonin neurons in the cord where there is an almost complete loss of amine distal to a transection. These results suggest that acetylcholine is contained primarily within neurons that are intrinsic to the cord as opposed to biogenic amine-containing neurons whose cell bodies are found in brain mesencephalon.

In another series of studies, Dr. Potter administered the neurotoxin 6-aminonicotinamide, which is known to produce morphological damage within the cord, and she found a significant fall of spinal cord acetylcholine. These studies provide suggestive evidence that cholinergic neurons are destroyed in the cord following treatment with this toxin.

Section on Molecular Pharmacodynamics (Chief, D.L. Cheney, Ph.D.)

I. Septal Nuclei: Organization and Interactions

With the help of specific immunohistochemical methods this section has studied histochemically the location of putative neuromodulators in various septal nuclei. To date we do not have a complete understanding of the functional interactions among various septal nuclei that regulate hippocampal functions. Nevertheless, much has been learned histologically and biochemically about the major connections of distinct areas of the septal complex and the putative transmitters operative in some of these pathways. The cholinergic septal-hippocampal pathway serves as a well-defined link between these two important structures of the limbic system. ACh turnover rate in the hippocampus has been shown to increase or decrease proportionally according to the activity of the cholinergic neurons originating in the septum. Moreover, these turnover rates have been shown to be modulated by intraseptal injections of agonists and antagonists of the various neurotransmitters or neuromodulators stored in various cell groups in the septum. By coupling this biochemical approach with techniques to study receptor organization in greater detail with regard to transmitter and cotransmitter interactions among the various

neuromodulators can be obtained.

Recent evidence has pointed to the existence of two classes of GABA receptors in the CNS: A GABA_A receptor toward which muscimol is an agonist and bicuculline is an antagonist, and a GABA_B receptor toward which baclofen is an agonist. The GABA_A receptor is thought to be the classical GABA receptor which is coupled to Cl⁻ channels and whose activation decreases cell firing. The GABA_B receptor is less well-characterized but has been implicated in the presynaptic inhibition of excitatory amino acid release. GABAergic drugs selective for either GABA_A or GABA_B sites were used in this study.

Since an involvement of the cholinergic septal hippocampal system in response inhibition is supported by a number of reports, Dr. Blaker (Staff Fellow) used the extinction of a food reinforced lever press response as a behavioral indicator of proper functioning of this cholinergic system. The turnover rate of acetylcholine in the hippocampus was used as a biochemical indicator of the cholinergic activity of septal projections to the hippocampus. In such a procedure, phosphoryl (³H)₂ choline was infused via the tail vein and the incorporation of the label into choline and acetylcholine was determined using a gas chromatography-mass fragmentography method.

From the choline and acetylcholine curves representing the change with time of the incorporation of the label, the fractional rate constant for acetylcholine efflux could be calculated. The fractional rate constant multiplied by the steady state concentration of acetylcholine determined the turnover rate of acetylcholine. The behavioral and biochemical effects of GABAergic drugs on the septal hippocampal system was studied by comparing the acetylcholine turnover rate (TR_{ACh}) in the rat hippocampus with extinction of a food reinforced lever press response after intraseptal injection of such drugs.

Muscimol, a GABA_A agonist, reproduced a dose dependent and simultaneous increase in responding during extinction and a decrease in TR_{ACh}. Bicuculline, a GABA_A antagonist, in equivalent doses had the opposite behavioral effect, i.e., a general decrease in responding. Baclofen, a GABA_B agonist, had behavioral effects similar to those produced by muscimol, but was 5-10 times more potent and did not alter the hippocampal TR_{ACh}.

Three to five weeks after bilateral injection of kainic acid into the hippocampus, there was a significant loss of bicuculline insensitive GABA binding (GABA_B sites) in the septum, but no loss of baclofen insensitive GABA binding (GABA_A sites). This loss is presumably due to the degeneration of the glutamatergic hippocampal pyramidal cells which have terminals in the lateral septum. We hypothesized that both muscimol and baclofen exert their behavioral effects by decreasing the functional output of the hippocampus, muscimol by decreasing the stimulatory input to the hippocampus, and baclofen by presynaptically inhibiting the hippocampal efferents to the septum.

Since it is known that the cholinergic connections from the medial septum to the hippocampus terminate on glutamatergic pyramidal cells which, in turn, project to the lateral septum, Dr., Blaker studied the neuronal localization of septal GABA_A and GABA_B binding sites. He injected kainic acid bilaterally into the hippocampus and allowed 3 to 5 weeks to complete the degeneration of hippocampal efferents. He then performed ³H-GABA binding assays on thoroughly washed septal membranes under conditions to distinguish specific binding to GABA_A and GABA_B sites. Kainic acid treatment was found to decrease GABA_B type binding by 40%, while having no effect on GABA_A binding.

This suggests that a sizable proportion of GABA_B sites in the septum are located presynaptically on afferents from the hippocampus. It thus seems probable that muscimol and baclofen both exert their behavioral effects by inhibiting the output in the hippocampus

of muscimol by inhibiting the stimulatory input to the hippocampus via the activation of GABA_A receptors and baclofen by presynaptically inhibiting hippocampal efferents via activation of GABA_B receptors.

II. Insecticides: Mechanisms of Toxicity in Mammals

Kepone is an insecticide which was the cause of an epidemic neurological syndrome as a result of an industrial pollution. We decided to study the cause of this toxicity in the rat model. Adult male rats receiving a single i.p. injection of kepone (80 mg/kg) exhibit tremors within a few hours following the injection. Since kepone-elicited tremors are relieved by injections of muscarinic receptor blockers, Dr. Gandolfi measured ACh turnover in various brain structures. He failed to detect evidence for an involvement of cholinergic presynaptic mechanisms in kepone toxicity.

Kepone inhibits turnover rate of GABA in striatum. Moreover, Dr. Gandolfi found that kepone down regulated 5HT₁ receptors and increased the turnover of 5HT in hippocampus and striatum. These results are compatible with the hypothesis that kepone decreases GABAergic tone; thereby indirectly it increases 5HT firing. Assuming that 5HT and ACh innervate the same pathway bringing antagonistic influences, the increase in 5HT tone may cause an increase in cholinergic tone of striatum causing tremors. In fact, we know that GABA neurons modulate raphe serotonergic nuclei.

Group on Physical Methods (J. Meek, Ph.D., Head)

The group on Physical Methods has utilized HPLC in studies of neurotransmitter turnover. Dr. Wroblewski (Visiting Fellow) initiated studies on glutamate turnover in the septum. Glutamate may be the most common excitatory transmitter in brain, but to date all attempts to distinguish "neurotransmitter glutamate" from metabolic glutamate have been unsuccessful. Drs. Wroblewski and Blaker were able to show that ornithine may be the precursor of the neurotransmitter pool. Evidence for the size of the pool and its half life were obtained from experiments combining lesions of the glutamatergic input to the septum with inhibition of ornithine aminotransferase with canaline. Experiments are now underway to confirm these findings using radiolabelled ornithine.

Dr. Giorgi (Visiting Fellow) followed up work begun earlier in this group on GABA turnover. He examined the interactions of various neurotransmitter agonists and antagonists on GABA turnover in rat striatum. He was able to demonstrate an increase in GABA turnover by glutamate, the putative neurotransmitter of the excitatory corticostriatal pathway. The effect was blocked by administration of a glutamate antagonist (GDEE). Despite attempts in many labs, the study of peptide turnover has been largely unsuccessful because of the presence of huge precursor molecules which contain multiple replicates of a number of peptides. Of course, the detection of the immediate precursor for a given neurotransmitter is almost impossible in these conditions.

Drs. Meek, Giorgi and Iadarola took the novel approach of using a posttranslation modification step (sulfation) to provide turnover information. They showed that sulfation of cholecystokinin (CCK-8) occurred after injection of ³⁵S-S₀ into cerebral cortex, and that the half life of disappearance of the CCK-8 could be readily measured. Drs. Giorgi and Meek are now examining the nature of the sulfation process. Discovery of a sulfotransferase inhibitor would not only allow for a convenient nonisotopic CCK turnover measurement, but also would provide a valuable pharmacological tool to study posttranslation processing proteins to peptides.

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